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(54) Title: HIGH MOLECULAR WEIGHT SURFACE PROTEINS OF NON-TYPEABLE HAEMOPHILUS (57) Abstract High molecular weight surface proteins of non-typeable <i>Haemophilus influenzae</i> which exhibit immunogenic properties and genes encoding the same are described. Specifically, genes coding for two immunodominant high molecular weight proteins, HMW1 and HMW2, have been cloned, expressed and sequenced, while genes coding for high molecular proteins HMW3 and HMW4 have also been cloned, expressed and sequenced.		

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TITLE OF INVENTION
HIGH MOLECULAR WEIGHT SURFACE PROTEINS
OF NON-TYPEABLE HAEMOPHILUS

FIELD OF INVENTION

5 This invention relates to high molecular weight proteins of non-typeable haemophilus.

BACKGROUND TO THE INVENTION

10 Non-typeable Haemophilus influenzae are non-encapsulated organisms that are defined by their lack of reactivity with antisera against known H. influenzae capsular antigens.

15 These organisms commonly inhabit the upper respiratory tract of humans and are frequently responsible for a variety of common mucosal surface infections, such as otitis media, sinusitis, conjunctivitis, chronic bronchitis and pneumonia. Otitis media remains an important health problem for children and most children have had at least one episode of otitis by their third birthday and approximately one-third of
20 children have had three or more episodes. Non-typeable Haemophilus influenzae generally accounts for about 20 to 25% of acute otitis media and for a larger percentage of cases of chronic otitis media with effusion.

25 A critical first step in the pathogenesis of these infections is colonization of the respiratory tract mucosa. Bacterial surface molecules which mediate adherence, therefore, are of particular interest as possible vaccine candidates.

30 Since the non-typeable organisms do not have a polysaccharide capsule, they are not controlled by the

present Haemophilus influenzae type b (Hib) vaccines, which are directed towards Hib bacterial capsular polysaccharides. The non-typeable strains, however, do produce surface antigens that can elicit bactericidal antibodies. Two of the major outer membrane proteins, P2 and P6, have been identified as targets of human serum bactericidal activity. However, it has been shown that the P2 protein sequence is variable, in particular in the non-typeable Haemophilus strains. Thus, a P2-based vaccine would not protect against all strains of the organism.

There have previously been identified by Barenkamp et al (Pediatr. Infect. Dis. J., 9:333-339, 1990) a group of high-molecular-weight (HMW) proteins of non-typeable Haemophilus influenzae that appeared to be major targets of antibodies present in human convalescent sera. Examination of a series of middle ear isolates revealed the presence of one or two such proteins in most strains. However, prior to the present invention, the structures of these proteins and their encoding nucleic acid sequences were unknown as were pure isolates of such proteins. In addition, the identification of surface accessible epitopes of such proteins was unknown.

SUMMARY OF INVENTION

The inventor, in an effort to further characterize the high molecular weight (HMW) non-typeable Haemophilus proteins, has cloned, expressed and sequenced the genes coding for two immunodominant HMW proteins (designated HMW1 and HMW2) from a prototype non-typeable Haemophilus strain and has cloned, expressed and sequenced the genes coding for two additional immunodominant HMW proteins (designated HMW3 and HMW4) from another non-typeable Haemophilus strain.

In accordance with one aspect of the present invention, therefore, there is provided an isolated and

purified nucleic acid molecule coding for a high molecular weight protein of a non-typeable Haemophilus strain, particularly a nucleic acid molecule coding for protein HMW1, HMW2, HMW3 or HMW4, as well as any variant or fragment of such protein which retains the immunological ability to protect against disease caused by a non-typeable Haemophilus strain.

The nucleic acid molecule may have a DNA sequence shown in Figure 1 (SEQ ID No: 1) and encoding HMW1 for strain 12 having the derived amino acid sequence of Figure 2 (SEQ ID No: 2). The nucleic acid molecule may have the DNA sequence shown in Figure 3 (SEQ ID No: 3) and encoding protein HMW2 for strain 12 having the derived amino acid sequence of Figure 4 (SEQ ID No: 4). The nucleic acid molecule may have the DNA sequence shown in Figure 8 (SEQ ID No: 7) and encoding HMW3 for strain 5 having the derived amino acid sequence of Figure 10 (SEQ ID No: 9). The nucleic acid molecule may have a DNA sequence shown in Figure 9 (SEQ ID No: 8) and encoding protein HMW4 for strain 5 having the derived amino acid sequence of Figure 10 (SEQ ID No: 10).

In another aspect of the invention, there is provided an isolated and purified nucleic acid molecule encoding a high molecular weight protein of a non-typeable Haemophilus strain, which is selected from the group consisting of:

- (a) a DNA sequence as shown in any one of Figures 1, 3, 8 and 9 (SEQ ID Nos: 1, 3, 7 and 8);
- (b) a DNA sequence encoding an amino acid sequence as shown in any one of Figures 2, 4 and 10 (SEQ ID Nos: 2, 4, 9 and 10); and
- (c) a DNA sequence which hybridizes under stringent conditions to any one of the sequences of (a) and (b).

A DNA sequence according to (c) may be one having at least about 90% identity of sequence to the DNA sequences (a) or (b).

5 The inventor has further found correct processing of the HMW protein requires the presence of additional downstream nucleic acid sequences. Accordingly, a further aspect of the present invention provides an isolated and purified gene cluster comprising a first nucleotide sequence encoding a high molecular weight
10 protein of a non-typeable Haemophilus strain and at least one downstream nucleotide sequence for effecting expression of a gene product of the first nucleotide sequence fully encoded by the structural gene.

15 The gene cluster may comprise a DNA sequence encoding high molecular weight protein HMW1 or HMW2 and two downstream accessory genes. The gene cluster may have the DNA sequence shown in Figure 6 (SEQ ID No: 5) or Figure 7 (SEQ ID No. 6).

20 In an additional aspect, the present invention includes a vector adapted for transformation of a host, comprising a nucleic acid molecule as provided herein, particularly the gene cluster provided herein. The vector may be an expression vector or a plasmid adapted for expression of the encoded high molecular weight
25 protein, fragments or analogs thereof, in a heterologous or homologous host and comprising expression means operatively coupled to the nucleic acid molecule. The expression means may include a nucleic acid portion encoding a leader sequence for secretion from the host of
30 the high molecular weight protein. The expression means may include a nucleic acid portion encoding a lipidation signal for expression from the host of a lipidated form of the high molecular weight protein. The host may be selected from, for example, E. coli, Bacillus,
35 Haemophilus, fungi, yeast, baculovirus and Semliki Forest Virus expression systems. The invention further includes

a recombinant high molecular weight protein of non-typeable Haemophilus or fragment or analog thereof producible by the transformed host.

5 In another aspect, the invention provides an isolated and purified high molecular weight protein of non-typeable Haemophilus influenzae which is encoded by a nucleic acid molecule as provided herein. Such high molecular weight proteins may be produced recombinantly to be devoid of non-high molecular weight proteins of
10 non-typeable Haemophilus influenzae or from natural sources.

Such protein may be characterized by at least one surface-exposed B-cell epitope which is recognized by monoclonal antibody AD6 (ATCC _____). Such protein may
15 be HMW1 encoded by the DNA sequence shown in Figure 1 (SEQ ID No: 1) and having the derived amino acid sequence of Figure 2 (SEQ ID No: 2) and having an apparent molecular weight of 125 kDa. Such protein may be HMW2 encoded by the DNA sequence shown in Figure 3 (SEQ ID No:
20 3) and having the derived amino acid sequence of Figure 4 (SEQ ID No: 4) and having an apparent molecular weight of 120 kDa. Such protein may be HMW3 encoded by the DNA sequence shown in Figure 8 (SEQ ID No: 7) and having the derived amino acid sequence of Figure 10 (SEQ ID No: 9)
25 and having an apparent molecular weight of 125 kDa. Such protein may be HMW4 encoded by the DNA sequence shown in Figure 9 (SEQ ID No: 8) and having the derived amino acid sequence shown in Figure 10 (SEQ ID No: 10) and having the apparent molecular weight of 123kDa.

30 A further aspect of the invention provides an isolated and purified high molecular weight protein of non-typeable Haemophilus influenzae which is antigenically related to the filamentous hemagglutinin surface protein of Bordetella pertussis, particularly
35 HMW1, HMW2, HMW3 or HMW4.

The novel high molecular weight proteins of non-typeable Haemophilus may be used as carrier molecules by linking to an antigen, hapten or polysaccharide for eliciting an immune response to the antigen, hapten or polysaccharide. An example of such polysaccharide is a protective polysaccharide against Haemophilus influenzae type b.

In a further aspect of the invention, there is provided a synthetic peptide having an amino acid sequence containing at least six amino acids and no more than 150 amino acids and corresponding to at least one protective epitope of a high molecular weight protein of non-typeable Haemophilus influenzae, specifically HMW1, HMW2, HMW3 or HMW4. The epitope may be one recognized by at least one of the monoclonal antibodies AD6 (ATCC ____) and 10C5 (ATCC ____). Specifically, the epitope may be located within 75 amino acids of the carboxy terminus of the HMW1 or HMW2 protein and recognized by the monoclonal antibody AD6.

The present invention also provides an immunogenic composition comprising an immunoeffective amount of an active component, which may be the novel high molecular weight protein or synthetic peptide provided herein, which may be formulated along with a pharmaceutically acceptable carrier therefor. The immunogenic composition may be formulated as a vaccine for *in vivo* administration to a host.

The immunogenic composition may be formulated as a microparticle, capsule, ISCOM or liposome preparation. The immunogenic composition may be used in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces. Some targeting molecules include vitamin B12 and fragments of bacterial toxins, as described in WO 92/17167 (Biotech Australia Pty. Ltd.), and monoclonal antibodies, as described in U.S. Patent No. 5,194,254 (Barber et al).

The immunogenic compositions of the invention (including vaccines) may further comprise at least one other immunogenic or immunostimulating material and the immunostimulating material may be at least one adjuvant.

5 Suitable adjuvants for use in the present invention include, (but are not limited to) aluminum phosphate, aluminum hydroxide, QS21, Quil A, derivatives and components thereof, ISCOM matrix, calcium phosphate, calcium hydroxide, zinc hydroxide, a glycolipid analog,
10 an octadecyl ester of an amino acid, a muramyl dipeptide polyphosphazare, ISCOMPRP, DC-chol, DBA and a lipoprotein and other adjuvants to induce a Th1 response. Advantageous combinations of adjuvants are described in
15 copending United States patent Application Serial No. 08/261,194 filed June 16, 1994, assigned to Connaught Laboratories Limited and the disclosure of which is incorporated herein by reference.

 In a further aspect of the invention, there is provided a method of generating an immune response in a
20 host, comprising administering thereto an immuno-effective amount of the immunogenic composition as provided herein. The immune response may be a humoral or a cell-mediated immune response. Hosts in which protection against disease may be conferred include
25 primates including humans.

 The present invention additionally provides a method of producing antibodies specific for a high molecular weight protein of non-typeable Haemophilus influenzae, comprising:

- 30 (a) administering the high molecular weight protein or epitope containing peptide provided herein to at least one mouse to produce at least one immunized mouse;
- (b) removing B-lymphocytes from the at least one immunized mouse;

(c) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;

(d) cloning the hybridomas;

5 (e) selecting clones which produce anti-high molecular weight protein antibody;

(f) culturing the anti-high molecular weight protein antibody-producing clones; and then

10 (g) isolating anti-high molecular weight protein antibodies from the cultures.

Additional aspects of the present invention include monoclonal antibody AD6 and monoclonal antibody 10C5.

The present invention provides, in an additional aspect thereof, a method for producing an immunogenic
15 composition, comprising administering the immunogenic composition provided herein to a first test host to determine an amount and a frequency of administration thereof to elicit a selected immune response against a high molecular weight protein of non-typeable Haemophilus
20 influenzae; and formulating the immunogenic composition in a form suitable for administration to a second host in accordance with the determined amount and frequency of administration. The second host may be a human.

The novel envelope protein provided herein is useful
25 in diagnostic procedures and kits for detecting antibodies to high molecular weight proteins of non-typeable Haemophilus influenzae. Further monoclonal antibodies specific for the high molecular protein or epitopes thereof are useful in diagnostic procedure and
30 kits for detecting the presence of the high molecular weight protein.

Accordingly, a further aspect of the invention provides a method of determining the presence in a sample, of antibodies specifically reactive with a high
35 molecular weight protein of Haemophilus influenzae comprising the steps of:

- 5 (a) contacting the sample with the high molecular weight protein or epitope-containing peptide as provided herein to produce complexes comprising the protein and any said antibodies present in the sample specifically reactive therewith; and
- (b) determining production of the complexes.

10 In a further aspect of the invention, there is provided a method of determining the presence, in a sample, of a high molecular weight protein of Haemophilus influenzae or an epitope-containing peptide, comprising the steps of:

- (a) immunizing a host with the protein or peptide as provided herein, to produce antibodies specific for the protein or peptide;
- 15 (b) contacting the sample with the antibodies to produce complexes comprising any high molecular weight protein or epitope-containing peptide present in the sample and said specific antibodies; and
- (c) determining production of the complexes.

20 A further aspect of the invention provides a diagnostic kit for determining the presence of antibodies in a sample specifically reactive with a high molecular weight protein of non-typeable Haemophilus influenzae or epitope-containing peptide, comprising:

- 25 (a) the high molecular weight protein or epitope-containing peptide as provided herein;
- (b) means for contacting the protein or peptide with the sample to produce complexes comprising the protein or peptide and any said antibodies present
- 30 in the sample; and
- (c) means for determining production of the complexes.

The invention also provides a diagnostic kit for detecting the presence, in a sample, of a high molecular

35 weight protein of Haemophilus influenzae or epitope-containing peptide, comprising:

(a) an antibody specific for the novel envelope protein as provided herein;

(b) means for contacting the antibody with the sample to produce a complex comprising the protein or peptide and protein-specific antibody; and

(c) means for determining production of the complex.

In this application, the term "high molecular weight protein" is used to define a family of high molecular weight proteins of Haemophilus influenzae, generally having an apparent molecular weight of from about 120 to about 130 kDa and includes proteins having variations in their amino acid sequences. In this application, a first protein or peptide is a "functional analog" of a second protein or peptide if the first protein or peptide is immunologically related to and/or has the same function as the second protein or peptide. The functional analog may be, for example, a fragment of the protein or a substitution, addition or deletion mutant thereof. The invention also extends to such functional analogs.

Advantages of the present invention include:

- an isolated and purified envelope high molecular weight protein of Haemophilus influenzae produced recombinantly to be devoid of non-high molecular weight proteins of Haemophilus influenzae or from natural sources as well as nucleic acid molecules encoding the same;
- high molecular weight protein specific human monoclonal antibodies which recognize conserved epitopes in such protein; and
- diagnostic kits and immunological reagents for specific identification of hosts infected by Haemophilus influenzae.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A to 1G contain the DNA sequence of a gene coding for protein HMW1 (SEQ ID No: 1). The hmw1A open reading frame extends from nucleotides 351 to 4958;

5 Figures 2A and 2B contain the derived amino acid sequence of protein HMW1 (SEQ ID No: 2);

Figures 3A to 3G contain the DNA sequence of a gene coding for protein HMW2 (SEQ ID No: 3). The open hmw2A open reading frame extends from nucleotides 382 to 4782;

10 Figures 4A and 4B contain the derived amino acid sequence of HMW2 (SEQ ID No: 4);

Figure 5A shows restriction maps of representative recombinant phages which contained the HMW1 or HMW2 structural genes and of HMW1 plasmid subclones. The shaded boxes indicate the location of the structural genes. In the recombinant phage, transcription proceeds from left to right for the HMW1 gene and from right to left for the HMW2 gene;

15 Figure 5B shows the restriction map of the T7 expression vector pT7-7. This vector contains the T7 RNA polymerase promoter $\phi 10$, a ribosomal binding site (rbs) and the translational start site for the T7 gene 10 protein upstream from a multiple cloning site;

20 Figures 6A to 6L contain the DNA sequence of a gene cluster for the hmw1 gene (SEQ ID NO: 5), comprising nucleotides 351 to 4958 (ORF a) (as in Figure 1), as well as two additional downstream genes in the 3' flanking region, comprising ORFs b, nucleotides 5114 to 6748 and c nucleotides 7062 to 9011;

25 Figures 7A to 7L contain the DNA sequence of a gene cluster for the hmw2 gene (SEQ ID NO: 6), comprising nucleotides 792 to 5222 (ORF a) (as in Figure 3), as well as two additional downstream genes in the 3' flanking region, comprising ORFs b, nucleotides 5375 to 7009, and c, nucleotides 7249 to 9198;

30

35

Figures 8A and 8B contain the DNA sequence of a gene coding for protein HMW3 (SEQ ID NO: 7);

Figures 9A and 9B contain the DNA sequence of a gene coding for protein HMW4 (SEQ ID NO: 8);

5 Figures 10A to 10L contain a comparison table for the derived amino acid sequence for proteins HMW1 (SEQ ID No: 2), HMW2 (SEQ ID No: 4), HMW3 (SEQ ID No: 9) and HMW4 (SEQ ID No: 10);

10 Figure 11 illustrates a Western immunoblot assay of phage lysates containing either the HMW1 or HMW2 recombinant proteins. Lysates were probed with an E. coli-absorbed adult serum sample with high-titer antibody against high molecular weight proteins. The arrows indicate the major immunoreactive bands of 125 and 120
15 kDa in the HMW1 and HMW2 lysates respectively;

 Figure 12 is a Western immunoblot assay of cell sonicates prepared from E. coli transformed with plasmid pT7-7 (lanes 1 and 2), pHMW1-2 (lanes 3 and 4), pHMW1-4 (lanes 5 and 6) or pHMW1-14 (lanes 7 and 8). The
20 sonicates were probed with an E. coli-absorbed adult serum sample with high-titer antibody against high-molecular weight proteins. Lanes labelled U and I sequence sonicates prepared before and after indication of the growing samples with IPTG, respectively. The
25 arrows indicate protein bands of interest as discussed below;

 Figure 13 is a graphical illustration of an ELISA with rHMW1 antiserum assayed against purified filamentous haemagglutinin of B. pertussis. Ab = antibody;

30 Figure 14 is a Western immunoblot assay of cell sonicates from a panel of epidemiologically unrelated non-typeable H. influenzae strains. The sonicates were probed with rabbit antiserum prepared against HMW1-4 recombinant protein. The strain designations are
35 indicated by the numbers below each line;

Figure 15 is a Western immunoblot assay of cell sonicates from a panel of epidemiologically unrelated non-typeable H. influenzae strains. The sonicates were probed with monoclonal antibody X3C, a murine IgG antibody which recognizes the filamentous hemagglutinin of B. pertussis. The strain designations are indicated by the numbers below each line;

Figure 16 shows an immunoblot assay of cell sonicates of non-typeable H. influenzae strain 12 derivatives. The sonicates were probed with rabbit antiserum prepared against HMW-1 recombinant protein. Lanes: 1, wild-type strain; 2, HMW2⁻ mutant; 3, HMW1⁻ mutant; 4. HMW1⁻ HMW2⁻ double mutant;

Figure 17 shows middle ear bacterial counts in PBS-immunized control animals (left panel) and HMW1/HMW2-immunized animals (right panel) seven days after middle ear inoculation with non-typeable Haemophilus influenzae strain 12. Data are log-transformed and the horizontal lanes indicate the means and standard deviations of middle ear fluid bacterial counts for only the infected animals in each group;

Figure 18 is a schematic diagram of pGEMEX[®]-hmw1 recombinant plasmids. The restriction enzymes are B-BamHI, E-EcoRI, C-ClaI, RV-EcoRV, Bst-BstEII and H-HindIII;

Figure 19 is a schematic diagram of pGEMEX[®]-hmw2 recombinant plasmids. The restriction enzymes are E-EcoRI, H-HindIII, Hc-HincII, M-MluI and X-XhoI;

Figure 20 is an immunoelectron micrograph of representative non-typeable Haemophilus influenzae strains after incubation with monoclonal antibody AD6 followed by incubation with goat anti-mouse IgG conjugated with 10-nm colloidal gold particles. Strains are: upper left panel-strain 12; upper right panel-strain 12 mutant deficient in expression of the high molecular

weight proteins; lower left panel-strain 5; lower right panel-strain 15;

Figure 21 is a Western immunoblot assay with Mab AD6 and HMW1 or HMW2 recombinant proteins. The upper left panel indicates the segments of hmw1A or hmw2A structural genes which are being expressed in the recombinant proteins. The lane numbers correspond to the indicated segments;

Figure 22 is a Western immunoblot assay with MAb 10C5 and HMW1 or HMW2 recombinant proteins. The upper panel indicates the segments of the hmw1A or hmw2A structural genes which are being expressed in the recombinant proteins. The lane numbers correspond to the indicated segments; and

Figure 23 is a Western immunoblot assay with MAb AD6 and a panel of unrelated non-typeable Haemophilus influenzae strains which express HMW1/HMW-2 like protein. Cell sonicates were prepared from freshly grown samples of each strain prior to analysis in the Western blot.

GENERAL DESCRIPTION OF INVENTION

The DNA sequences of the genes coding for the HMW1 and HMW2 proteins of non-typeable Haemophilus influenzae strain 12, shown in Figures 1 and 3 respectively, were shown to be about 80% identical, with the first 1259 base pairs of the genes being identical. The open reading frame extend from nucleotides 351 to 4958 and from nucleotide 382 to 4782 respectively. The derived amino acid sequences of the two HMW proteins, shown in Figures 2 and 4 respectively, are about 70% identical. Furthermore, the encoded proteins are antigenically related to the filamentous hemagglutinin surface protein of Bordetella pertussis. A monoclonal antibody prepared against filamentous hemagglutinin (FHA) of Bordetella pertussis was found to recognize both of the high molecular weight proteins. This data suggests that the

5 HMW and FHA proteins may serve similar biological functions. The derived amino acid sequences of the HMW1 and HMW2 proteins show sequence similarity to that for the FHA protein. It has further been shown that these
10 antigenically-related proteins are produced by the majority of the non-typeable strains of Haemophilus. Antisera raised against the protein expressed by the HMW1 gene recognizes both the HMW2 protein and the B. pertussis FHA. The present invention includes an isolated and purified high molecular weight protein of non-typeable haemophilus which is antigenically related to the B. pertussis FHA and which may be obtained from natural sources or produced recombinantly.

15 A phage genomic library of a known strain of non-typeable Haemophilus was prepared by standard methods and the library was screened for clones expressing high molecular weight proteins, using a high titre antiserum against HMW's. A number of strongly reactive DNA clones were plaque-purified and sub-cloned into a T7 expression
20 plasmid. It was found that they all expressed either one or the other of the two high-molecular-weight proteins designated HMW1 and HMW2, with apparent molecular weights of 125 and 120 kDa, respectively, encoded by open reading frames of 4.6 kb and 4.4 kb, respectively.

25 Representative clones expressing either HMW1 or HMW2 were further characterized and the genes isolated, purified and sequenced. The DNA sequence of HMW1 is shown in Figure 1 and the corresponding derived amino acid sequence in Figure 2. Similarly, the DNA sequence of
30 HMW2 is shown in Figure 3 and the corresponding derived amino acid sequence in Figure 4. Partial purification of the isolated proteins and N-terminal sequence analysis indicated that the expressed proteins are truncated since their sequence starts at residue number 442 of both full
35 length HMW1 and HMW2 gene products.

Subcloning studies with respect to the hmw1 and hmw2 genes indicated that correct processing of the HMW proteins required the products of additional downstream genes. It has been found that both the hmw1 and hmw2 genes are flanked by two additional downstream open reading frames (ORFs), designated b and c, respectively, (see Figures 6 and 7).

The b ORFs are 1635 bp in length, extending from nucleotides 5114 to 6748 in the case of hmw1 and nucleotides 5375 to 7009 in the case of hmw2, with their derived amino acid sequences being 99% identical. The derived amino acid sequences demonstrate similarity with the derived amino acid sequences of two genes which encode proteins required for secretion and activation of hemolysins of P. mirabilis and S. marcescens.

The c ORFs are 1950 bp in length, extending from nucleotides 7062 to 9011 in the case of hmw1 and nucleotides 7249 to 9198 in the case of hmw2, with their derived amino acid sequences 96% identical. The hmw1 c ORF is preceded by a series of 9 bp direct tandem repeats. In plasmid subclones, interruption of the hmw1 b or c ORF results in defective processing and secretion of the hmw1 structural gene product.

The two high molecular weight proteins HMW1 and HMW2 have been isolated and purified by the procedures described below in the Examples and shown to be protective against otitis media in chinchillas and to function as adhesins. These results indicate the potential for use of such high molecular proteins and structurally-related proteins of other non-typeable strains of Haemophilus influenzae as components in immunogenic compositions for protecting a susceptible host, such as a human infant, against disease caused by infection with non-typeable Haemophilus influenzae.

Since the proteins provided herein are good cross-reactive antigens and are present in the majority

of non-typeable Haemophilus strains, it is evident that these HMW proteins may become integral constituents of a universal Haemophilus vaccine. Indeed, these proteins may be used not only as protective antigens against otitis, sinusitis and bronchitis caused by the non-typeable Haemophilus strains, but also may be used as carriers for the protective Hib polysaccharides in a conjugate vaccine against meningitis. The proteins also may be used as carriers for other antigens, haptens and polysaccharides from other organisms, so as to induce immunity to such antigens, haptens and polysaccharides.

The nucleotide sequences encoding two high molecular weight proteins of a different non-typeable Haemophilus strain (designated HMW3 and HMW4), namely strain 5 have been elucidated, and are presented in Figures 8 and 9 (SEQ ID Nos: 7 and 8). HMW3 has an apparent molecular weight of 125 kDa while HMW4 has an apparent molecular weight of 123 kDa. These high molecular weight proteins are antigenically related to the HMW1 and HMW2 proteins and to FHA. Figure 10 contains a multiple sequence comparison of the derived amino acid sequences for the four high molecular weight proteins identified herein (HMW1, SEQ ID No: 2; HMW2, SEQ ID No: 4; HMW3, SEQ ID No: 9; HMW4, SEQ ID No. 10). As may be seen from this comparison, stretches of identical amino acid sequence may be found throughout the length of the comparison, with HMW3 more closely resembling HMW1 and HMW4 more closely resembling HMW2. This information is highly suggestive of a considerable sequence homology between high molecular weight proteins from various non-typeable Haemophilus strains. This information is also suggestive that the HMW3 and HMW4 proteins will have the same immunological properties as the HMW1 and HMW2 proteins and that corresponding HMW proteins from other non-typeable Haemophilus strains will have the same immunological properties as the HMW1 and HMW2 proteins.

In addition, mutants of non-typeable H. influenzae strains that are deficient in expression of HMW1 or HMW2 or both have been constructed and examined for their capacity to adhere to cultured human epithelial cells. The hmw1 and hmw2 gene clusters have been expressed in E. coli and have been examined for in vitro adherence. The results of such experimentation, described below, demonstrate that both HMW1 and HMW2 mediate attachment and hence are adhesins and that this function is present even in the absence of other H. influenzae surface structures. The ability of a bacterial surface protein to function as an adhesin provides strong in vitro evidence for its potential role as a protective antigen. In view of the considerable sequence homology between the HMW3 and HMW4 proteins and the HMW1 and HMW2 proteins, these results indicate that HMW3 and HMW4 also are likely to function as adhesins and that other HMW proteins of other strains of non-typeable Haemophilus influenzae similarly are likely to function as adhesins. This expectation is borne out by the results described in the Examples below.

With the isolation and purification of the high molecular weight proteins, the inventor is able to determine the major protective epitopes of the proteins by conventional epitope mapping and synthesizing peptides corresponding to these determinants for incorporation into fully synthetic or recombinant vaccines. Accordingly, the invention also comprises a synthetic peptide having at least six and no more than 150 amino acids and having an amino acid sequence corresponding to at least one protective epitope of a high molecular weight protein of a non-typeable Haemophilus influenzae. Such peptides are of varying length that constitute portions of the high molecular weight proteins, that can be used to induce immunity, either directly or as part of a conjugate, against the respective organisms and thus

constitute active components of immunogenic compositions for protection against the corresponding diseases.

In particular, the applicant has sought to identify regions of the high molecular weight proteins which are demonstrated experimentally to be surface-exposed B-cell epitopes and which are common to all or at least a large number of non-typeable strains of Haemophilus influenzae. The strategy which has been adopted by the inventor has been to:

- 10 (a) generate a panel of monoclonal antibodies reactive with the high molecular weight proteins;
- (b) screen those monoclonal antibodies for reactivity with surface epitopes of intact bacteria using immunoelectron microscopy or other suitable screening technique;
- 15 (c) map the epitopes recognized by the monoclonal antibody by determining the reactivity of the monoclonals with a panel of recombinant fusion proteins; and
- 20 (d) determining the reactivity of the monoclonal antibodies with heterologous non-typable Haemophilus influenzae strains using standard Western blot assays.

Using this approach, the inventor has identified one monoclonal antibody, designated AD6 (ATCC _____), which recognized a surface-exposed B-cell epitope common to all non-typeable H. influenzae which express the HMW1 and HMW2 proteins. The epitope recognized by this antibody was mapped to a 75 amino acid sequence at the carboxy termini of both HMW1 and HMW2 proteins. The ability to identify shared surface-exposed epitopes on the high molecular weight adhesion proteins suggests that it would be possible to develop recombinant or synthetic peptide based vaccines which would be protective against disease caused by the majority of non-typeable Haemophilus influenzae.

The present invention also provides any variant or fragment of the proteins that retains the potential immunological ability to protect against disease caused by non-typeable Haemophilus strains. The variants may be constructed by partial deletions or mutations of the genes and expression of the resulting modified genes to give the protein variants.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis, treatment of bacterial infections and the generation of immunological reagents. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the high molecular weight proteins of Haemophilus influenzae, as well as analogs and fragments thereof, and synthetic peptides containing epitopes of the protein, as disclosed herein. The immunogenic composition elicits an immune response which produces antibodies, including anti-high molecular weight protein antibodies and antibodies that are opsonizing or bactericidal.

Immunogenic compositions, including vaccines, may be prepared as injectables, as liquid solutions or emulsions. The active component may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously or

intramuscularly. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkylene glycols or triglycerides. Oral formulations may include normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 1 to 95% of the active component. The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the HMW proteins. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host.

The concentration of the active component in an immunogenic composition according to the invention is in

general about 1 to 95%. A vaccine which contains antigenic material of only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

Immunogenicity can be significantly improved if the antigens are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in

increasing antibody responses to diphtheria and tetanus toxoids is well established and a HBsAg vaccine has been adjuvanted with alum. While the usefulness of alum is well established for some applications, it has limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

To efficiently induce humoral immune responses (HIR) and cell-mediated immunity (CMI), immunogens are often emulsified in adjuvants. Many adjuvants are toxic, inducing granulomas, acute and chronic inflammations (Freund's complete adjuvant, FCA), cytolysis (saponins and Pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (LPS and MDP). Although FCA is an excellent adjuvant and widely used in research, it is not licensed for use in human or veterinary vaccines because of its toxicity.

Desirable characteristics of ideal adjuvants include:

- (1) lack of toxicity;
- (2) ability to stimulate a long-lasting immune response;
- (3) simplicity of manufacture and stability in long-term storage;
- (4) ability to elicit both CMI and HIR to antigens administered by various routes, if required;

- (5) synergy with other adjuvants;
(6) capability of selectively interacting with populations of antigen presenting cells (APC);
(7) ability to specifically elicit appropriate T_H1 or
5 T_H2 cell-specific immune responses; and
(8) ability to selectively increase appropriate antibody isotype levels (for example, IgA) against antigens.

U.S. Patent No. 4,855,283 granted to Lockhoff et al on August 8, 1989 which is incorporated herein by
10 reference thereto teaches glycolipid analogues including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Thus, Lockhoff et al. (US Patent No.
15 4,855,283 and ref. 29) reported that N-glycolipid analogs displaying structural similarities to the naturally-occurring glycolipids, such as glycosphingolipids and glyco glycerolipids, are capable of eliciting strong immune responses in both herpes simplex virus vaccine and
20 pseudorabies virus vaccine. Some glycolipids have been synthesized from long chain-alkylamines and fatty acids that are linked directly with the sugars through the anomeric carbon atom, to mimic the functions of the naturally occurring lipid residues.

U.S. Patent No. 4,258,029 granted to Moloney, incorporated herein by reference thereto, teaches that octadecyl tyrosine hydrochloride (OTH) functioned as an adjuvant when complexed with tetanus toxoid and formalin inactivated type I, II and III poliomyelitis virus
25 vaccine. Also, Nixon-George et al. (ref. 30), reported that octadecyl esters of aromatic amino acids complexed with a recombinant hepatitis B surface antigen, enhanced the host immune responses against hepatitis B virus.
30

Lipidation of synthetic peptides has also been used
35 to increase their immunogenicity. Thus, Wiesmuller 1989, describes a peptide with a sequence homologous to a foot-

and-mouth disease viral protein coupled to an adjuvant tripalmityl-s-glyceryl-cysteinylserylserine, being a synthetic analogue of the N-terminal part of the lipoprotein from Gram negative bacteria. Furthermore, 5 Deres et al. 1989, reported in vivo priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine which comprised of modified synthetic peptides derived from influenza virus nucleoprotein by linkage to a lipopeptide, N-palmityl-s-[2,3-10 bis(palmitylxy)-(2RS)-propyl-[R]-cysteine (TPC).

2. Immunoassays

The high molecular weight protein of Haemophilus influenzae of the present invention is useful as an immunogen for the generation of anti-protein antibodies, 15 as an antigen in immunoassays including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art for the detection of antibodies. In ELISA assays, the protein is immobilized onto a selected surface, for 20 example, a surface capable of binding proteins, such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed protein, a nonspecific protein, such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral 25 with regard to the test sample, may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

30 The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This may include diluting the sample with diluents, such as solutions of BSA, 35 bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to

incubate for from about 2 to 4 hours, at temperatures such as of the order of about 25° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound protein, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined by subjecting the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody is an antibody having specificity for human immunoglobulins and in general IgG. To provide detecting means, the second antibody may have an associated activity such as an enzymatic activity that will generate, for example, a colour development upon incubating with an appropriate chromogenic substrate. Quantification may then be achieved by measuring the degree of colour generation using, for example, a visible spectra spectrophotometer.

3. Use of Sequences as Hybridization Probes

The nucleotide sequences of the present invention, comprising the sequences of the genes encoding the high molecular weight proteins of specific strains of non-typeable Haemophilus influenzae, now allow for the identification and cloning of the genes from any species of non-typeable Haemophilus and other strains of non-typeable Haemophilus influenzae.

The nucleotide sequences comprising the sequences of the genes of the present invention are useful for their ability to selectively form duplex molecules with complementary stretches of other genes of high molecular weight proteins of non-typeable Haemophilus. Depending on the application, a variety of hybridization conditions may be employed to achieve varying degrees of selectivity

of the probe toward the other genes. For a high degree of selectivity, relatively stringent conditions are used to form the duplexes, such as low salt and/or high temperature conditions, such as provided by 0.02 M to 0.15 M NaCl at temperatures of between about 50°C to 70°C. For some applications, less stringent hybridization conditions are required such as 0.15 M to 0.9 M salt, at temperatures ranging from between about 20°C to 55°C. Hybridization conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be a method of choice depending on the desired results. In general, convenient hybridization temperatures in the presence of 50% formamide are: 42°C for a probe which is 95 to 100% homologous to the target fragment, 37°C for 90 to 95% homology and 32°C for 85 to 90% homology.

In a clinical diagnostic embodiment, the nucleic acid sequences of the genes of the present invention may be used in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of providing a detectable signal. In some diagnostic embodiments, an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of a radioactive tag may be used. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with samples containing gene sequences encoding high molecular weight proteins of non-typeable Haemophilus.

The nucleic acid sequences of genes of the present invention are useful as hybridization probes in solution

hybridizations and in embodiments employing solid-phase procedures. In embodiments involving solid-phase procedures, the test DNA (or RNA) from samples, such as clinical samples, including exudates, body fluids (e. g., serum, amniotic fluid, middle ear effusion, sputum, bronchoalveolar lavage fluid) or even tissues, is adsorbed or otherwise affixed to a selected matrix or surface. The fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes comprising the nucleic acid sequences of the genes or fragments thereof of the present invention under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required depending on, for example, the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe etc. Following washing of the hybridization surface so as to remove non-specifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label. As with the selection of peptides, it is preferred to select nucleic acid sequence portions which are conserved among species of non-typeable Haemophilus. The selected probe may be at least about 18 bp and may be in the range of about 30 bp to about 90 bp long.

4. Expression of the High Molecular Weight Protein Genes

Plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell may be used for the expression of the genes encoding high molecular weight proteins of non-typeable Haemophilus in expression systems. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* may be transformed using pBR322 which contains genes for ampicillin and tetracycline resistance and thus provides

easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, promoters which can be used by the host cell for expression of its own proteins.

In addition, phage vectors containing replicon and control sequences that are compatible with the host can be used as a transforming vector in connection with these hosts. For example, the phage in lambda GEMTM-11 may be utilized in making recombinant phage vectors which can be used to transform host cells, such as E. coli LE392.

Promoters commonly used in recombinant DNA construction include the β -lactamase (penicillinase) and lactose promoter systems (Chang et al., 1978; Itakura et al., 1977; Goeddel et al., 1979; Goeddel et al., 1980) and other microbial promoters such as the T7 promoter system (U.S. Patent 4,952,496). Details concerning the nucleotide sequences of promoters are known, enabling a skilled worker to ligate them functionally with genes. The particular promoter used will generally be a matter of choice depending upon the desired results. Hosts that are appropriate for expression of the genes encoding the high molecular weight proteins, fragment analogs or variants thereof, include E. coli, Bacillus species, Haemophilus, fungi, yeast or the baculovirus expression system may be used.

In accordance with this invention, it is preferred to make the high molecular weight proteins by recombinant methods, particularly since the naturally occurring high molecular weight protein as purified from a culture of a species of non-typeable Haemophilus may include trace amounts of toxic materials or other contaminants. This problem can be avoided by using recombinantly produced proteins in heterologous systems which can be isolated from the host in a manner to minimize contaminants in the purified material. Particularly desirable hosts for

expression in this regard include Gram positive bacteria which do not have LPS and are, therefore, endotoxin free. Such hosts include species of Bacillus and may be particularly useful for the production of non-pyrogenic high molecular weight protein, fragments or analogs thereof. Furthermore, recombinant methods of production permit the manufacture of HMW1, HMW2, HMW3 or HMW4, and corresponding HMW proteins from other non-typeable Haemophilus influenzae strains, or fragments thereof, separate from one another and devoid of non-HMW protein of non-typeable Haemophilus influenzae.

Biological Deposits

Certain hybridomas producing monoclonal antibodies specific for high molecular weight protein of Haemophilus influenzae according to aspects of the present invention that are described and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 12301 Parklawn Drive, Rockville, Maryland, USA, 20852, pursuant to the Budapest Treaty and prior to the filing of this application. Samples of the deposited hybridomas will become available to the public upon grant of a patent based upon this United States patent application. The invention described and claimed herein is not to be limited in scope by the hybridomas deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or similar hybridomas that produce similar or equivalent antibodies as described in this application are within the scope of the invention.

Deposit Summary

<u>Hybridomas</u>	<u>ATCC Designation</u>	<u>Date Deposited</u>
AD6		
10C5		

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry, and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1:

This Example describes the isolation of DNA encoding HMW1 and HMW2 proteins, cloning and expression of such proteins, and sequencing and sequence analysis of the DNA molecules encoding the HMW1 and HMW2 proteins.

Non-typeable H.influenzae strains 5 and 12 were isolated in pure culture from the middle ear fluid of children with acute otitis media. Chromosomal DNA from strain 12, providing genes encoding proteins HMW1 and HMW2, was prepared by preparing Sau3A partial restriction digests of chromosomal DNA and fractionating on sucrose gradients. Fractions containing DNA fragments in the 9 to 20 kbp range were pooled and a library was prepared by ligation into λ EMBL3 arms. Ligation mixtures were packaged in vitro and plate-amplified in a P2 lysogen of E. coli LE392.

For plasmid subcloning studies, DNA from a representative recombinant phage was subcloned into the T7 expression plasmid pT7-7, containing the T7 RNA polymerase promoter ϕ 10, a ribosome-binding site and the

translational start site for the T7 gene 10 protein upstream from a multiple cloning site (see Figure 5B).

DNA sequence analysis was performed by the dideoxy method and both strands of the HMW1 gene and a single strand of the HMW2 gene were sequenced.

Western immunoblot analysis was performed to identify the recombinant proteins being produced by reactive phage clones (Figure 11). Phage lysates grown in LE392 cells or plaques picked directly from a lawn of LE392 cells on YT plates were solubilized in gel electrophoresis sample buffer prior to electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 7.5% or 11% polyacrylamide modified Laemmli gels. After transfer of the proteins to nitrocellulose sheets, the sheets were probed sequentially with an E. coli-absorbed human serum sample containing high-titer antibody to the high-molecular-weight proteins and then with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) second antibody. Sera from healthy adults contains high-titer antibody directed against surface-exposed high-molecular-weight proteins of non-typeable H. influenzae. One such serum sample was used as the screening antiserum after having been extensively absorbed with LE392 cells.

To identify recombinant proteins being produced by E. coli transformed with recombinant plasmids, the plasmids of interest were used to transform E. coli BL21 (DE3)/pLySS. The transformed strains were grown to an A_{600} of 0.5 in L broth containing 50 μ g of ampicillin per ml. IPTG was then added to 1 mM. One hour later, cells were harvested, and a sonicate of the cells was prepared. The protein concentrations of the samples were determined by the bicinchoninic acid method. Cell sonicates containing 100 μ g of total protein were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and transferred to

nitrocellulose. The nitrocellulose was then probed sequentially with the E. coli-absorbed adult serum sample and then with alkaline phosphatase-conjugated goat anti-human IgG second antibody.

5 Western immunoblot analysis also was performed to determine whether homologous and heterologous non-typeable H. influenzae strains expressed high-molecular-weight proteins antigenically related to the protein encoded by the cloned HMW1 gene (rHMW1). Cell sonicates
10 of bacterial cells were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose. Nitrocellulose was probed sequentially with polyclonal rabbit rHMW1 antiserum and then with alkaline
15 phosphatase-conjugated goat anti-rabbit IgG second antibody.

Finally, Western immunoblot analysis was performed to determine whether non-typeable Haemophilus strains expressed proteins antigenically related to the
20 filamentous hemagglutinin protein of Bordetella pertussis. Monoclonal antibody X3C, a murine immunoglobulin G (IgG) antibody which recognizes filamentous hemagglutinin, was used to probe cell sonicates by Western blot. An alkaline phosphatase-
25 conjugated goat anti-mouse IgG second antibody was used for detection.

To generate recombinant protein antiserum, E. coli BL21(DE3)/pLyss was transformed with pHMW1-4, and expression of recombinant protein was induced with IPTG,
30 as described above. A cell sonicate of the bacterial cells was prepared and separated into a supernatant and pellet fraction by centrifugation at 10,000 x g for 30 min. The recombinant protein fractionated with the pellet fraction. A rabbit was subcutaneously immunized
35 on biweekly schedule with 1 mg of protein from the pellet fraction, the first dose given with Freund's complete

adjuvant and subsequent doses with Freund's incomplete adjuvant. Following the fourth injection, the rabbit was bled. Prior to use in the Western blot assay, the antiserum was absorbed extensively with sonicates of the host E. coli strain transformed with cloning vector alone.

To assess the sharing of antigenic determinants between HMW1 and filamentous hemagglutinin, enzyme-linked immunosorbent assay (ELISA) plates (Costar, Cambridge, Mass.) were coated with 60 μ l of a 4- μ g/ml solution of filamentous hemagglutinin in Dulbecco's phosphate-buffered saline per well for 2 h at room temperature. Wells were blocked for 1 h with 1% bovine serum albumin in Dulbecco's phosphate-buffered saline prior to addition of serum dilutions. rHMW1 antiserum was serially diluted in 0.1% Brij (Sigma, St. Louis, Mo.) in Dulbecco's phosphate-buffered saline and incubated for 3 h at room temperature. After being washed, the plates were incubated with peroxidase-conjugated goat anti-rabbit IgG antibody (Bio-Rad) for 2 h at room temperature and subsequently developed with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) at a concentration of 0.54 in mg/ml in 0.1 M sodium citrate buffer, pH 4.2, containing 0.03% H₂O₂. Absorbances were read on an automated ELISA reader.

Recombinant phage expressing HMW1 or HMW2 were recovered as follows. The non-typeable H. influenzae strain 12 genomic library was screened for clones expressing high-molecular-weight proteins with an E. coli-absorbed human serum sample containing a high titer of antibodies directed against the high-molecular-weight proteins.

Numerous strongly reactive clones were identified along with more weakly reactive ones. Twenty strongly reactive clones were plaque-purified and examined by Western blot for expression of recombinant proteins.

Each of the strongly reactive clones expressed one of two types of high-molecular-weight proteins, designated HMW1 and HMW2. The major immunoreactive protein bands in the HMW1 and HMW2 lysates migrated with apparent molecular masses of 125 and 120 kDa, respectively. In addition to the major bands, each lysate contained minor protein bands of higher apparent molecular weight. Protein bands seen in the HMW2 lysates at molecular masses of less than 120 kDa were not regularly observed and presumably represent proteolytic degradation products. Lysates of LE392 infected with the λ EMBL3 cloning vector alone were non-reactive when immunologically screened with the same serum sample. Thus, the observed activity was not due to cross-reactive *E. coli* proteins or λ EMBL3-encoded proteins. Furthermore, the recombinant proteins were not simply binding immunoglobulin nonspecifically, since the proteins were not reactive with the goat anti-human IgG conjugate alone, with normal rabbit sera, or with serum from a number of healthy young infants.

Representative clones expressing either the HMW1 or HMW2 recombinant proteins were characterized further. The restriction maps of the two phage types were different from each other, including the regions encoding the HMW1 and HMW2 structural genes. Figure 5A shows restriction maps of representative recombinant phage which contained the HMW1 or HMW2 structural genes. The locations of the structural genes are indicated by the shaded bars.

HMW1 plasmid subclones were constructed by using the T7 expression plasmid T7-7 (Fig. 5A and B). HMW2 plasmid subclones also were constructed, and the results with these latter subclones were similar to those observed with the HMW1 constructs.

The approximate location and direction of transcription of the HMW1 structure gene were initially determined by using plasmid pHMW1 (Fig. 5A). This

plasmid was constructed by inserting the 8.5-kb BamHI-SalI fragment from λ HMW1 into BamHI- and SalI-cut pT7-7. E. coli transformed with pHMW1 expressed an immunoreactive recombinant protein with an apparent
5 molecular mass of 115 kDa, which was strongly inducible with IPTG. This protein was significantly smaller than the 125-kDa major protein expressed by the parent phage, indicating that it either was being expressed as a fusion protein or was truncated at the carboxy terminus.

10 To more precisely localize the 3' end of the structural gene, additional plasmids were constructed with progressive deletions from the 3' end of the pHMW1 construct. Plasmid pHMW1-1 was constructed by digestion of pHMW1 with PstI, isolation of the resulting 8.8-kb
15 fragment, and religation. Plasmid pHMW1-2 was constructed by digestion of pHMW1 with HindIII, isolation of the resulting 7.5-kb fragment, and religation. E. coli transformed with either plasmid pHMW1-1 or pHMW1-2 also expressed an immunoreactive recombinant protein with
20 an apparent molecular mass of 115 kDa. These results indicated that the 3' end of the structural gene was 5' of the HindIII site. Figure 12 demonstrates the Western blot results with pHMW1-2 transformed cells before and after IPTG indicates (lanes 3 and 4, respectively). The
25 115 kDa recombinant protein is indicated by the arrow. Transformants also demonstrated cross-reactive bands of lower apparent molecular weight, and probably represent partial degradation products. Shown for comparison and the results for E. coli transformed with the pT7-7
30 cloning vector alone (Fig. 12, lanes 1 and 2).

To more precisely localize the 5' end of the gene, plasmids pHMW1-4 and pHMW1-7 were constructed. Plasmid pHMW1-4 was constructed by cloning the 5.1-kb BamHI-HindIII fragment from λ HMW1 into a pT7-7-derived plasmid
35 containing the upstream 3.8-kb EcoRI-BamHI fragment. E. coli transformed with pHMW1-4 expressed an immunoreactive

protein with an apparent molecular mass of approximately 160 kDa (Fig. 12, lane 6). Although protein production was inducible with IPTG, the levels of protein production in these transformants were substantially lower than those with the pHMW1-2 transformants described above. Plasmid pHMW1-7 was constructed by digesting pHMW1-4 with NdeI and SpeI. The 9.0-kbp fragment generated by this double digestion was isolated, blunt ended, and religated. E. coli transformed with pHMW1-7 also expressed an immunoreactive protein with an apparent molecular mass of 160 kDa, a protein identical in size to that expressed by the pHMW1-4 transformants. The result indicated that the initiation codon for the HMW1 structural gene was 3' of the SpeI site. DNA sequence analysis (described below) confirmed this conclusion.

As noted above, the λ HMW1 phage clones expressed a major immunoreactive band of 125 kDa, whereas the HMW1 plasmid clones pHMW1-4 and pHMW1-7, which contained what was believed to be the full-length gene, expressed an immunoreactive protein of approximately 160 kDa. This size discrepancy was disconcerting. One possible explanation was that an additional gene or genes necessary for correct processing of the HMW1 gene product were deleted in the process of subcloning. To address this possibility, plasmid pHMW1-14 was constructed. This construct was generated by digesting pHMW1 with NdeI and MluI and inserting the 7.6-kbp NdeI-MluI fragment isolated from pHMW1-4. Such a construct would contain the full-length HMW1 gene as well as the DNA 3' of the HMW1 gene which was present in the original HMW1 phage. E. coli transformed with this plasmid expressed major immunoreactive proteins with apparent molecular masses of 125 and 160 kDa as well as additional degradation products (Fig. 12, lanes 7 and 8). The 125- and 160-kDa bands were identical to the major and minor immunoreactive bands detected in the HMW1 phage lysates.

Interestingly, the pHMW1-14 construct also expressed significant amounts of protein in the uninduced condition, a situation not observed with the earlier constructs.

5 The relationship between the 125- and 160-kDa proteins remains somewhat unclear. Sequence analysis, described below, reveals that the HMW1 gene would be predicted to encode a protein of 159 kDa. It is believed that the 160-kDa protein is a precursor form of the
10 mature 125-kDa protein, with the conversion from one protein to the other being dependent on the products of the two downstream genes.

Sequence analysis of the HMW1 gene (Figure 1) revealed a 4,608-bp open reading frame (ORF), beginning
15 with an ATG codon at nucleotide 351 and ending with a TAG stop codon at nucleotide 4959. A putative ribosome-binding site with the sequence AGGAG begins 10 bp upstream of the putative initiation codon. Five other in-frame ATG codons are located within 250 bp of the
20 beginning of the ORF, but none of these is preceded by a typical ribosome-binding site. The 5'-flanking region of the ORF contains a series of direct tandem repeats, with the 7-bp sequence ATCTTTC repeated 16 times. These tandem repeats stop 100 bp 5' of the putative initiation
25 codon. An 8-bp inverted repeat characteristic of a rho-independent transcriptional terminator is present, beginning at nucleotide 4983, 25 bp 3' of the presumed translational stop. Multiple termination codons are present in all three reading frames both upstream and
30 downstream of the ORF. The derived amino acid sequence of the protein encoded by the HMW1 gene (Figure 2) has a molecular weight of 159,000, in good agreement with the apparent molecular weights of the proteins expressed by the HMW1-4 and HMW1-7 transformants. The derived amino
35 acid sequence of the amino terminus does not demonstrate the characteristics of a typical signal sequence. The

BamHI site used in generation of pHMW1 comprises bp 1743 through 1748 of the nucleotide sequence. The ORF downstream of the BamHI site would be predicted to encode a protein of 111 kDa, in good agreement with the 115 kDa estimated for the apparent molecular mass of the pHMW1-encoded fusion protein.

The sequence of the HMW2 gene (Figure 3) consists of a 4,431-bp ORF, beginning with an ATG codon at nucleotide 352 and ending with a TAG stop codon at nucleotide 4783. The first 1,259 bp of the ORF of the HMW2 gene are identical to those of the HMW1 gene. Thereafter, the sequences begin to diverge but are 80% identical overall. With the exception of a single base addition at nucleotide 93 of the HMW2 sequence, the 5'-flanking regions of the HMW1 and HMW2 genes are identical for 310 bp upstream from the respective initiation codons. Thus, the HMW2 gene is preceded by the same set of tandem repeats and the same putative ribosome-binding site which lies 5' of the HMW1 gene. A putative transcriptional terminator identical to that identified 3' of the HMW1 ORF is noted, beginning at nucleotide 4804. The discrepancy in the lengths of the two genes is principally accounted for by a 186-bp gap in the HMW2 sequence, beginning at nucleotide position 3839. The derived amino acid sequence of the protein encoded by the HMW2 gene (Figure 4) has a molecular weight of 155,000 and is 71% identical with the derived amino acid sequence of the HMW1 gene.

The derived amino acid sequences of both the HMW1 and HMW2 genes (Figures 2 and 4) demonstrated sequence similarity with the derived amino acid sequence of filamentous hemagglutinin of Bordetella pertussis, a surface-associated protein of this organism. The initial and optimized TFASTA scores for the HMW1-filamentous hemagglutinin sequence comparison were 87 and 186, respectively, with a word size of 2. The z score for the

comparison was 45.8. The initial and optimized TFASTA scores for the HMW2-filamentous hemagglutinin sequence comparison were 68 and 196, respectively. The z score for the latter comparison was 48.7. The magnitudes of the initial and optimized TFASTA scores and the z scores suggested that a biologically significant relationship existed between the HMW1 and HMW2 gene products and filamentous hemagglutinin. When the derived amino acid sequences of HMW1, HMW2, and filamentous hemagglutinin genes were aligned and compared, the similarities were most notable at the amino-terminal ends of the three sequences. Twelve of the first 22 amino acids in the predicted peptide sequences were identical. In addition, the sequences demonstrated a common five-amino-acid stretch, Asn-Pro-Asn-Gly-Ile, and several shorter stretches of sequence identity within the first 200 amino acids.

Example 2:

This Example describes the relationship of filamentous hemagglutinin and the HMW1 protein.

To further explore the HMW1-filamentous hemagglutinin relationship, the ability of antiserum prepared against the HMW1-4 recombinant protein (rHMW1) to recognize purified filamentous hemagglutinin was assessed (Figure 13). The rHMW1 antiserum demonstrated ELISA reactivity with filamentous hemagglutinin in a dose-dependent manner. Preimmune rabbit serum had minimal reactivity in this assay. The rHMW1 antiserum also was examined in a Western blot assay and demonstrated weak but positive reactivity with purified filamentous hemagglutinin in this system also.

To identify the native Haemophilus protein corresponding to the HMW1 gene product and to determine the extent to which proteins antigenically related to the HMW1 cloned gene product were common among other non-typeable H. influenzae strains, a panel of Haemophilus

strains was screened by Western blot with the rHMW1 antiserum. The antiserum recognized both a 125- and a 120-kDa protein band in the homologous strain 12 (Figure 14), the putative mature protein products of the HMW1 and HMW2 genes, respectively. The 120-kDa protein appears as a single band in Figure 14, wherein it appeared as a doublet in the HMW2 phage lysates (Figure 11).

When used to screen heterologous non-typeable H. influenzae strains, rHMW1 antiserum recognized high-molecular-weight proteins in 75% of 125 epidemiologically unrelated strains. In general, the antiserum reacted with one or two protein bands in the 100- to 150-kDa range in each of the heterologous strains in a pattern similar but not identical to that seen in the homologous strain (Figure 14).

Monoclonal antibody X3C is a murine IgG antibody directed against the filamentous hemagglutinin protein of B. pertussis. This antibody can inhibit the binding of B. pertussis cells to Chinese hamster ovary cells and HeLa cells in culture and will inhibit hemagglutination of erythrocytes by purified filamentous hemagglutinin. A Western blot assay was performed in which this monoclonal antibody was screened against the same panel of non-typeable H. influenzae strains discussed above (Figure 14). Monoclonal antibody X3C recognized both the high-molecular-weight proteins in non-typeable H. influenzae strain 12 which were recognized by the recombinant-protein antiserum (Figure 15). In addition, the monoclonal antibody recognized protein bands in a subset of heterologous non-typeable H. influenzae strains which were identical to those recognized by the recombinant-protein antiserum, as may be seen by comparison of Figures 14 and 15. On occasion, the filamentous hemagglutinin monoclonal antibody appeared to recognize only one of the two bands which had been recognized by the recombinant-protein antiserum (compare

strain lane 18 in Figures 14 and 15, for example). Overall, monoclonal antibody X3C recognized high-molecular-weight protein bands identical to those recognized by the rHMW1 antiserum in approximately 35% of our collection of non-typeable H. influenzae strains.

Example 3:

This Example describes the adhesin properties of the HMW1 and HMW2 proteins.

Mutants deficient in expression of HMW1, HMW2 or both proteins were constructed to examine the role of these proteins in bacterial adherence. The following strategy was employed. pHMW1-14 (see Example 1, Figure 5A) was digested with BamHI and then ligated to a kanamycin cassette isolated on a 1.3-kb BamHI fragment from pUC4K. The resultant plasmid (pHMW1-17) was linearized by digestion with XbaI and transformed into non-typeable H. influenzae strain 12, followed by selection for kanamycin resistant colonies. Southern analysis of a series of these colonies demonstrated two populations of transformants, one with an insertion in the HMW1 structural gene and the other with an insertion in the HMW2 structural gene. One mutant from each of these classes was selected for further studies.

Mutants deficient in expression of both proteins were recovered using the following protocol. After deletion of the 2.1-kb fragment of DNA between two EcoRI sites spanning the 3'-portion of the HMW1 structural gene and the 5'-portion of a downstream gene encoding an accessory processing protein in pHMW-15, the kanamycin cassette from pUC4K was inserted as a 1.3-kb EcoRI fragment. The resulting plasmid (pHMW1-16) was linearized by digestion with XbaI and transformed into strain 12, followed again by selection for kanamycin resistant colonies. Southern analysis of a representative sampling of these colonies demonstrated that in seven of eight cases, insertion into both the

HMW1 and HMW2 loci had occurred. One such mutant was selected for further studies.

To confirm the intended phenotypes, the mutant strains were examined by Western blot analysis with a polyclonal antiserum against recombinant HMW1 protein. The parental strain expressed both the 125-kD HMW1 and the 120-kD HMW2 protein (Figure 16). In contrast, the HMW2⁻ mutant failed to express the 120-kD protein, and the HMW1 mutant failed to express the 125-kD protein. The double mutant lacked expression of either protein. On the basis of whole cell lysates, outer membrane profiles, and colony morphology, the wild type strain and the mutants were otherwise identical with one another. Transmission electron microscopy demonstrated that none of the four strains expressed pili.

The capacity of wild type strain 12 to adhere to Chang epithelial cells was examined. In such assays, bacteria were inoculated into broth and allowed to grow to a density of $\sim 2 \times 10^9$ cfu/ml. Approximately 2×10^7 cfu were inoculated onto epithelial cell monolayers, and plates were gently centrifuged at $165 \times g$ for 5 minutes to facilitate contact between bacteria and the epithelial surface. After incubation for 30 minutes at 37°C in 5% CO₂, monolayers were rinsed 5 times with PBS to remove nonadherent organisms and were treated with trypsin-EDTA (0.05% trypsin, 0.5% EDTA) in PBS to release them from the plastic support. Well contents were agitated, and dilutions were plated on solid medium to yield the number of adherent bacteria per monolayer. Percent adherence was calculated by dividing the number of adherent cfu per monolayer by the number of inoculated cfu.

As depicted in Table 1 below (the Tables appear at the end of the descriptive text), this strain adhered quite efficiently, with nearly 90% of the inoculum binding to the monolayer. Adherence by the mutant expressing HMW1 but not HMW2 (HMW2⁻) was also quite

efficient and comparable to that by the wild type strain. In contrast, attachment by the strain expressing HMW2 but deficient in expression of HMW1 (HMW1⁻) was decreased about 15-fold relative to the wild type. Adherence by the double mutant (HMW1⁻/HMW2⁻) was decreased even further, approximately 50-fold compared with the wild type and approximately 3-fold compared with the HMW1 mutant. Considered together, these results suggest that both the HMW1 protein and the, HMW2 protein influence attachment to Chang epithelial cells. Interestingly, optimal adherence to this cell line appears to require HMW1 but not HMW2.

Example 4:

This Example illustrates the preparation and expression of HMW3 and HMW4 proteins and their function as adhesins.

Using the plasmids pHMW1-16 and pHMW1-17 (see Example 3) and following a scheme similar to that employed with strain 12 as described in Example 3, three non-typeable Haemophilus strain 5 mutants were isolated, including one with the kanamycin gene inserted into the hmw1-like (designated hmw3) locus, a second with an insertion in the hmw2-like (designated hmw4) locus, and a third with insertions in both loci. As predicted, Western immunoblot analysis demonstrated that the mutant with insertion of the kanamycin cassette into the hmw1-like locus had lost expression of the HMW3 125-kD protein, while the mutant with insertion into the hmw2-like locus failed to express the HMW4 123-kD protein. The mutant with a double insertion was unable to express either of the high molecular weight proteins.

As shown in Table 1 below, wild type strain 5 demonstrated high level adherence, with almost 80% of the inoculum adhering per monolayer. Adherence by the mutant deficient in expression of the HMW2-like protein (i.e. HMW4 protein) was also quite high. In contrast,

adherence by the mutant unable to express the HMW1-like protein (i.e. HMW3 protein) was reduced about 5-fold relative to the wild type, and attachment by the double mutant was diminished even further (approximately 25-fold). Examination of Giemsa-stained samples confirmed these observations (not shown). Thus, the results with strain 5 for proteins HMW3 and HMW4 corroborate the findings with strain 12 and the HMW1 and HMW2 proteins.

Example 5:

This Example contains additional data concerning the adhesin properties of the HMW1 and HMW2 proteins.

To confirm an adherence function for the HMW1 and HMW2 proteins and to examine the effect of HMW1 and HMW2 independently of other *H. influenzae* surface structures, the hmw1 and the hmw2 gene clusters were introduced into *E. coli* DH5 α , using plasmids pHMW1-14 and pHMW2-21, respectively. As a control, the cloning vector, pT7-7, was also transformed into *E. coli* DH5 α . Western blot analysis demonstrated that *E. coli* DH5 α containing the hmw1 genes expressed a 125 kDa protein, while the same strain harboring the hmw2 genes expressed a 120-kDa protein. *E. coli* DH5 α containing pT7-7 failed to react with antiserum against recombinant HMW1. Transmission electron microscopy revealed no pili or other surface appendages on any of the *E. coli* strains.

Adherence by the *E. coli* strains was quantitated and compared with adherence by wild type non-typeable *H. influenzae* strain 12. As shown in Table 2 below, adherence by *E. coli* DH5 α containing vector alone was less than 1% of that for strain 12. In contrast, *E. coli* DH5 α harboring the hmw1 gene cluster demonstrated adherence levels comparable to those for strain 12. Adherence by *E. coli* DH5 α containing the hmw2 genes was approximately 6-fold lower than attachment by strain 12 but was increased 20-fold over adherence by *E. coli* DH5 α with pT7-7 alone. These results indicate that the HMW1

and HMW2 proteins are capable of independently mediating attachment to Chang conjunctival cells. These results are consistent with the results with the H. influenzae mutants reported in Examples 3 and 4, providing further evidence that, with Chang epithelial cells, HMW1 is a more efficient adhesin than is HMW2.

Experiments with E. coli HB101 harboring pT7-7, pHMW1-14, or pHMW2-21 confirmed the results obtained with the DH5 α derivatives (see Table 2).

Example 6:

This Example illustrates the copurification of HMW1 and HMW2 proteins from wild-type non-typeable H. influenzae strain.

HMW1 and HMW2 were isolated and purified from non-typeable H. influenzae (NTHI) strain 12 in the following manner. Non-typeable Haemophilus bacteria from frozen stock culture were streaked onto a chocolate plate and grown overnight at 37°C in an incubator with 5% CO₂. 50ml starter culture of brain heart infusion (BHI) broth, supplemented with 10 μ g/ml each of hemin and NAD was inoculated with growth on chocolate plate. The starter culture was grown until the optical density (O.D. - 600nm) reached 0.6 to 0.8 and then the bacteria in the starter culture was used to inoculate six 500 ml flasks of supplemented BHI using 8 to 10 ml per flask. The bacteria were grown in 500 ml flasks for an additional 5 to 6 hours at which time the O.D. was 1.5 or greater. Cultures were centrifuged at 10,000 rpm for 10 minutes.

Bacterial pellets were resuspended in a total volume of 250 ml of an extraction solution comprising 0.5 M NaCl, 0.01 M Na₂EDTA, 0.01 M Tris 50 μ M 1,10-phenanthroline, pH 7.5. The cells were not sonicated or otherwise disrupted. The resuspended cells were allowed to sit on ice at 0°C for 60 minutes. The resuspended cells were centrifuged at 10,000 rpm for 10 minutes at 4°C to remove the majority of intact cells and cellular

debris. The supernatant was collected and centrifuged at 100,000 x g for 60 minutes at 4°C. The supernatant again was collected and dialyzed overnight at 4°C against 0.01 M sodium phosphate, pH 6.0.

5 The sample was centrifuged at 10,000 rpm for 10 minutes at 4°C to remove insoluble debris precipitated from solution during dialysis. The supernatant was applied to a 10 ml CM Sepharose column which has been pre-equilibrated with 0.01 M sodium phosphate, pH 6.
10 Following application to this column, the column was washed with 0.01 M sodium phosphate. Proteins were elevated from the column with a 0 - 0.5M KCl gradient in 0.01 M Na phosphate, pH 6 and fractions were collected for gel examination. Coomassie gels of column fractions
15 were carried out to identify those fractions containing high molecular weight proteins. The fractions containing high molecular weight proteins were pooled and concentrated to a 1 to 3 ml volume in preparation for application of sample to gel filtration column.

20 A Sepharose CL-4B gel filtration column was equilibrated with phosphate-buffered saline, pH 7.5. The concentrated high molecular weight protein sample was applied to the gel filtration column and column fractions were collected. Coomassie gels were performed on the
25 column fractions to identify those containing high molecular weight proteins. The column fractions containing high molecular weight proteins were pooled.

Example 7:

30 This Example illustrates the use of specified HMW1 and HMW2 proteins in immunization studies.

 The copurified HMW1 and HMW2 proteins prepared as described in Example 6 were tested to determine whether they would protect against experimental otitis media caused by the homologous strain.

35 Healthy adult chinchillas, 1 to 2 years of age with weights of 350 to 500g, received three monthly

subcutaneous injections with 40 μ g of an HMW1-HMW2 protein mixture in Freund's adjuvant. Control animals received phosphate-buffered saline in Freund's adjuvant. One month after the last injection, the animals were
5 challenged by intrabullar inoculation with 300 cfu of NTHI strain 12.

Middle ear infection developed in 5 of 5 control animals versus 5 of 10 immunized animals. Although only 5 of 10 chinchillas were protected in this test, the test
10 conditions are very stringent, requiring bacteria to be injected directly into the middle ear space and to proliferate in what is in essence a small abscess cavity. As seen from the additional data below, complete protection of chinchillas can be achieved.

The five HMW1/HMW2-immunized animals that did not
15 develop otitis media demonstrated no signs of middle ear inflammation when examined by otoscopy nor were middle ear effusions detectable.

Among the five HMW1/HMW2-immunized animals that
20 became infected, the total duration of middle ear infection as assessed by the persistence of culture-positive middle ear fluid was not different from controls. However, the degree of inflammation of the tympanic membranes was subjectively less than in the
25 HMW1/HMW2-immunized animals. When quantitative bacterial counts were performed on the middle ear fluid specimens recovered from infected animals, notable differences were apparent between the HMW1/HMW2-immunized and PBS-immunized animals (Figure 17). Shown in Figure 17 are
30 quantitative middle ear fluid bacterial counts from animals on day 7 post-challenge, a time point associated with the maximum colony counts in middle ear fluid. The data were log-transformed for purpose of statistical comparison. The data from the control animals are shown
35 on the left and data from the high molecular weight protein immunized animals on the right. The two

horizontal lines indicate the respective means and standard derivations of middle ear fluid colony counts for only the infected animals in each group. As can be seen from this Figure, the HMW1/HMW2-immunized animals had significantly lower middle ear fluid bacterial counts than the PBS-immunized controls, geometric means of 7.4×10^6 and 1.3×10^5 , respectively ($p=0.02$, Students' t-test)

Serum antibody titres following immunization were comparable in uninfected and infected animals. However, infection in immunized animals was uniformly associated with the appearance of bacteria down-regulated in expression of the HMW proteins, suggesting bacterial selection in response to immunologic pressure.

Although this data shows that protection following immunization was not complete, this data suggests the HMW adhesin proteins are potentially important protective antigens which may comprise one component of a multi-component NTHI vaccine.

In addition, complete protection has been achieved in the chinchilla model at lower dosage challenge, as set forth in Table 3 below.

Groups of five animals were immunized with 20 μ g of the HMW1-HMW2 mixture prepared as described in Example 6 on days 1, 28 and 42 in the presence of alum. Blood samples were collected on day 53 to monitor the antibody response. On day 56, the left ear of animals was challenged with about 10 cfu of H. influenzae strain 12. Ear infection was monitored on day 4. Four animals in Group 3 were infected previously by H. influenzae strain 12 and were recovered completely for at least one month before the second challenge.

Example 8:

This Example illustrates the provision of synthetic peptides corresponding to a portion only of the HMW1 protein.

A number of synthetic peptides were derived from HMW1. Antisera then were raised to these peptides. The anti-peptide antisera to peptide HMW1-P5 was shown to recognize HMW1. Peptide HMW1-P5 covers amino acids 1453 to 1481 of HMW1, has the sequence VDEVIEAKRILEKVKDLSDEEREALAKLG (SEQ ID No: 11), and represents bases 1498 to 1576 in Figure 10.

This finding demonstrates that the DNA sequence and the derived protein is being interpreted in the correct reading frame and that peptides derived from the sequence can be produced which will be immunogenic.

Example 9:

This Example describes the generation of monoclonal antibodies to the high molecular weight proteins of non-typeable H. influenzae.

Monoclonal antibodies were generated using standard techniques. In brief, female BALB/c mice (4 to 6 weeks old) were immunized by intraperitoneal injection with high molecular weight proteins purified from nontypable Haemophilus strain 5 or strain 12, as described in Example 6. The first injection of 40 to 50 μ g of protein was administered with Freund's complete adjuvant and the second dose, received four to five weeks after the first, was administered with phosphate-buffered saline. Three days following the second injection, the mice were sacrificed and splenic lymphocytes were fused with SP2/0-Ag14 plasmacytoma cells.

Two weeks following fusion, hybridoma supernatants were screened for the presence of high molecular weight protein specific antibodies by a dot-blot assay. Purified high molecular weight proteins at a concentration of 10 μ g per ml in TRIS-buffered saline (TBS), were used to sensitize nitrocellulose sheets (Bio-Rad Laboratories, Richmond, CA) by soaking for 20 minutes. Following a blocking step with TBS-3% gelatin, the nitrocellulose was incubated for 60 minutes at room

temperature with individual hybridoma supernatants, at a 1:5 dilution in TBS-0.1 % Tween, using a 96-well Bio-Dot micro-filtration apparatus (Bio-Rad). After washing, the sheets were incubated for one hour with alkaline-phosphatase-conjugated affinity isolated goat-anti(mouse IgG + IgM) antibodies (Tago, Inc., Burlingame, CA). Following additional washes, positive supernatants were identified by incubation of the nitrocellulose sheet in alkaline phosphatase buffer (0.10 M TRIS, 0.10 M NaCl, 0.005 M MgCl₂,) containing nitroblue tetrazolium (0.1 mg/ml) and 5-bromo-4-chloro-3-indoyl phosphate (BCIP) (0.05 mg/ml).

For the antibody isotyping and immunoelectron microscopy studies to be described below, the monoclonal antibodies were purified from hybridoma supernatants. The antibodies recovered in this work were all of the IgG class. To purify the monoclonal antibodies, the hybridoma supernatants were first subjected to ammonium sulfate precipitation (50% final concentration at 0°C). Following overnight incubation, the precipitate was recovered by centrifugation and resolubilized in phosphate buffered saline. The solution was then dialyzed overnight against 0.01 M sodium phosphate buffer, pH 6.0. The following day the sample was applied to a DEAE-Sephacel column preequilibrated with the same phosphate buffer and the proteins were subsequently eluted with a KCl gradient. Column fractions containing the monoclonal antibodies were identified by examination of samples on Coomassie gels for protein bands typical of light and heavy chains.

The isotype of each monoclonal antibody was determined by immunodiffusion using the Ouchterlony method. Immunodiffusion plates were prepared on glass slides with 10 ml of 1% DNA-grade agarose (FMC Bioproducts, Rockland, ME) in phosphate-buffered saline. After the agarose solidified, 5-mm wells were punched

into the agarose in a circular pattern. The center well contained a concentrated preparation of the monoclonal antibody being evaluated and the surrounding wells contained goat anti-mouse subclass-specific antibodies (Tago). The plates were incubated for 48 hours in a humid chamber at 4°C and then examined for white lines of immunoprecipitation.

Hybridoma supernatants which were reactive in the dot-blot assay described above were examined by Western blot analysis, both to confirm the reactivity with the high molecular weight proteins of the homologous nontypable Haemophilus strain and to examine the cross-reactivity with similar proteins in heterologous strains. Nontypable Haemophilus influenzae cell sonicates containing 100 µg of total protein were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis on 7.5% acrylamide gels, and transferred to nitrocellulose using a Genie electrophoretic blotter (Idea Scientific Company, Corvallis, OR) for 45 min at 24 V. After transfer, the nitrocellulose sheet was blocked and then probed sequentially with the hybridoma supernatant, with alkaline phosphatase-conjugated goat-anti(mouse IgG + IgM) second antibody, and finally bound antibodies were detected by incubation with nitroblue tetrazolium/BCIP solution. This same assay was employed to examine the reactivity of the monoclonals with recombinant fusion proteins expressed in *E. coli* (see below).

In preparation for immunoelectronmicroscopy, bacteria were grown overnight on supplemented chocolate agar and several colonies were suspended in phosphate-buffered-saline containing 1 % albumin. A 20-µl drop of this bacterial suspension was then applied to a carbon-coated grid and incubated for 2 min. Excess fluid was removed and the specimen was then incubated for 5 min with the purified high molecular weight protein-specific

monoclonal antibody being analyzed. Following removal of excess liquid and a wash with phosphatebuffered saline, the specimen was incubated with anti-mouse IgG conjugated to 10-nm colloidal gold particles. Following final washes with phosphate-buffered saline, the sample was rinsed with distilled water. Staining of the bacterial cells was performed with 0.5% uranyl acetate for 1 min. Samples were then examined in a Phillips 201c electron microscope.

Fourteen different hybridomas were recovered which produced monoclonal antibodies reactive with the purified HMW1 and HMW2 proteins of nontypable Haemophilus strain 12 in the immunoblot screening assay. Of the monoclonals screened by immunoelectron microscopy to date, as described below, two were demonstrated to bind surface epitopes on prototype strain 12. These two monoclonal antibodies, designated AD6 (ATCC _____) and 10C5 (ATCC _____), were both of the IgG1 subclass.

Example 10:

This Example describes the identification of surface-exposed B-cell epitopes of high molecular weight proteins of non-typeable H. influenzae.

To map epitopes recognized by the monoclonal antibodies, their reactivity with a panel of recombinant fusion proteins expressed by pGEMEX® recombinant plasmids was examined. These plasmids were constructed by cloning various segments of the hmw1a or hmw2A structural genes into T7 expression vectors pGEMEX® -1 and GEMEX®-2 (Promega Corporation, Madison, WI). Shown in Figures 18 and 19 are the schematic diagrams depicting the segments derived from the hmw1 and hmw2 gene clusters cloned into the pGEMEX® expression plasmids. These segments were inserted such that in-frame fusions were created at each junction site. Thus, these plasmids encode recombinant fusion proteins containing pGEMEX®-encoded T7 gene 10 amino acids in the regions indicated by the hatched bars

and hmw1a or hmw2A encoded amino acids in the regions indicated by the black bars in these Figures. A stop codon is present at the junction of the black and white segments of each bar.

5 Four discrete sites within the hmw1A structural gene were selected as the 5' ends of the hmw1 inserts. For each 5' end, a series of progressively smaller inserts was created by taking advantage of convenient downstream restriction sites. The first recombinant plasmid
10 depicted in Figure 18 was constructed by isolating a 4.9 kbp BamHI-HindIII fragment from pHMW1-14 (Example 1, Figure 5A), which contains the entire hmw1 gene cluster and inserting it into BamHI-HindIII digested pGEMEX®-1. The second recombinant plasmid in this set was
15 constructed by digesting the "parent" plasmid with BstEII-HindIII, recovering the 6.8 kbp larger fragment, blunt-ending with Klenow DNA polymerase, and religating. The third recombinant plasmid in this set was constructed by digesting the "parent" plasmid with ClaI-HindIII,
20 recovering the 6.0 kbp larger fragment, blunt-ending, and religating. The next set of four hmw1 recombinant plasmids was derived from a "parent" plasmid constructed by ligating a 2.2 kbp EcoRI fragment from the hmw1 gene cluster into EcoRI-digested pGEMEX®-2. The other three
25 recombinant plasmids in this second set were constructed by digesting at downstream BstEII, EcoRV, and ClaI sites, respectively, using techniques similar to those just described. The third set of three recombinant plasmids depicted was derived from a "parent" plasmid constructed
30 by double-digesting the first recombinant plasmid described above (i.e. the one containing the 4.9 kbp BamHI-HindIII fragment) with BamHI and ClaI, blunt-ending, and religating. This resulted in a construct encoding a recombinant protein with an in-frame fusion at
35 the ClaI site of the hmw1A gene. The remaining two plasmids in this third set were constructed by digesting

at downstream BstEII and EcoRV sites, respectively. Finally, the fourth set of two recombinant plasmids was derived from a "parent" plasmid constructed by double-digesting the original BamHI-HindIII construct with
5 HincII and EcoRV, then religating. This resulted in a construct encoding a recombinant protein with an in-frame fusion at the EcoRV site of the hmw1A gene. The remaining plasmid in this fourth set was constructed by digesting at the downstream BstEII site.

10 Three discrete sites with the hmw2A structural gene were selected as the 5' ends of the hmw2 inserts. The first recombinant plasmid depicted in Figure 19 was constructed by isolating a 6.0 kbp EcoRI-XhoI fragment from pHMW2-21, which contains the entire hmw2 gene
15 cluster, and inserting it into EcoRI-SalI digested pGEMEX®-1. The second recombinant plasmid in this set was constructed by digesting at an MluI site near the 3' end of the hmw2A gene. The second set of two hmw2 recombinant plasmids was derived from a "parent" plasmid
20 constructed by isolating a 2.3 kbp HindIII fragment from pHMW2-21 and inserting it into HindIII-digested pGEMEX®-2. The remaining plasmid in this second set was constructed by digesting at the downstream MluI site. Finally, the last plasmid depicted was constructed by
25 isolating a 1.2 kbp HincII-HindIII fragment from the indicated location in the hmw2 gene cluster and inserting it into HincII-HindIII digested pGEMEX®-1.

Each of the recombinant plasmids was used to transform E. coli strain JM101. The resulting
30 transformants were used to generate the recombinant fusion proteins employed in the mapping studies. To prepare recombinant proteins, the transformed E. coli strains were grown to an A_{600} of 0.5 in L broth containing 50 μ g of ampicillin per ml. IPTG was then added to 1mM
35 and mGPl-2, the M13 phage containing the T7 RNA polymerase gene, was added at multiplicity of infection

of 10. One hour later, cells were harvested, and a sonicate of the cells was prepared. The protein concentrations of the samples were determined and cell sonicates containing 100 μ g of total protein were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and examined on Coomassie gels to assess the expression level of recombinant fusion proteins. Once high levels of expression of the recombinant fusion proteins were confirmed, the cell sonicates were used in the Western blot analyses described above.

Shown in Figure 20 is an electron micrograph demonstrating surface binding of Mab AD6 to representative nontypable Haemophilus influenzae strains. In the upper left panel of the Figure is nontypable Haemophilus strain 12 and in the upper right panel is a strain 12 derivative which no longer expressed the high molecular weight proteins. As can be seen, colloidal gold particles decorate the surface of strain 12, indicating bound AD6 antibody on the surface. In contrast, no gold particles are evident on the surface of the strain 12 mutant which no longer expresses the high molecular weight proteins. These results indicate that monoclonal antibody AD6 is recognizing a surface-exposed epitope on the high molecular weight proteins of strain 12. Analogous studies were performed with monoclonal antibody 10C5 demonstrating it too bound to surface-accessible epitopes on the high molecular weight HMW1 and HMW2 proteins of strain 12.

Having identified two surface-binding monoclonals, the epitope which each monoclonal recognized was mapped. To accomplish this task, the two sets of recombinant plasmids containing various portions of either the hmw1a or hmw2A structural genes (Figures 18 and 19) were employed. With these complementary sets of recombinant plasmids, the epitopes recognized by the monoclonal

antibodies were mapped to relatively small regions of the very large HMW1 and HMW2 proteins.

To localize epitopes recognized by Mab AD6, the pattern of reactivity of this monoclonal antibody with a large set of recombinant fusion protein was examined. Figure 21 is a Western blot which demonstrates the pattern of reactivity of Mab AD6 with five recombinant fusion proteins, a relevant subset of the larger number originally examined. From analysis of the pattern of reactivity of Mab AD6 with this set of proteins, one is able to map the epitope it recognizes to a very short segment of the HMW1 and HMW2 proteins. A brief summary of this analysis follows. For reference, the relevant portions of the hmw1A or hmw2A structural genes which were expressed in the recombinant proteins being examined are indicated in the diagram at the top of the figure. As shown in lane 1, Mab AD6 recognizes an epitope encoded by fragment 1, a fragment which encompasses the distal one-fourth of the hmw1A gene. Reactivity is lost when only the portion of the gene comprising fragment 2 is expressed. This observation localizes the AD6 epitope somewhere within the last 180 amino acids at the carboxy-terminal end of the HMW1 protein. Mab AD6 also recognizes an epitope encoded by fragment 3, derived from the hmw2A structural gene. This is a rather large fragment which encompasses nearly one-third of the gene. Reactivity is lost when fragment 4 is expressed. The only difference between fragments 3 and 4 is that the last 225 base pairs at the 3' end of the hmw2A structural gene were deleted in the latter construct. This observation indicates that the AD6 epitope is encoded by this short terminal segment of the hmw2A gene. Strong support for this idea is provided by the demonstrated binding of Mab AD6 to the recombinant protein encoded by fragment 5, a fragment encompassing the distal one-tenth of the hmw2A structural gene. Taken together, these data

identify the AD6 epitope as common to both the HMW1 and HMW2 proteins and place its location with 75 amino acids of the carboxy termini of the two proteins.

Figure 22 is a Western blot demonstrating the pattern of reactivity of Mab 10C5 with the same five recombinant fusion proteins examined in Figure 21. As shown in lane 1, Mab 10C5 recognizes an epitope encoded by fragment 1. In contrast to Mab AD6, Mab 10C5 also recognizes an epitope encoded by fragment 2. Also in contrast to Mab AD6, Mab 10C5 does not recognize any of the hmw2A-derived recombinant fusion proteins. Thus, these data identify the 10C5 epitope as being unique to the HMW1 protein and as being encoded by the fragment designated as fragment 2 in this figure. This fragment corresponds to a 155-amino acid segment encoded by the EcoRV-BstEII segment of the hmw1A structural gene.

Having identified the approximate locations of the epitopes on HMW1 and HMW2 recognized by the two monoclonals, the extent to which these epitopes were shared by the high molecular weight proteins of heterologous nontypable Haemophilus strains was next determined. When examined in Western blot assays with bacterial cell sonicates, Mab AD6 was reactive with epitopes expressed on the high molecular weight proteins of 75% of the inventor's collection of more than 125 nontypable Haemophilus influenzae strains. In fact, this monoclonal appeared to recognize epitopes expressed on high molecular weight proteins in virtually all nontypable Haemophilus strains which we previously identified as expressing HMW1/HMW2-like proteins. Figure 23 is an example of a Western blot demonstrating the reactivity of Mab AD6 with a representative panel of such heterologous strains. As can be seen, the monoclonal antibody recognizes one or two bands in the 100 to 150 kDa range in each of these strains. For reference, the strain shown in lane 1 is prototype strain 12 and the two

bands visualized represent HMW1 and HMW2 as the upper and lower immunoreactive bands, respectively.

5 In contrast to the broad cross-reactivity observed with Mab AD6, Mab 10C5 was much more limited in its ability to recognize high molecular weight proteins in heterologous strains. Mab 10C5 recognized high molecular weight proteins in approximately 40% of the strains which expressed HMW1/HMW2-like proteins. As was the case with Mab AD6, Mab 10C5 did not recognize proteins in any the
10 nontypable Haemophilus strains which did not express HMW1/HMW2-like proteins.

In a limited fashion, the reactivity of Mab AD6 with surface-exposed epitopes on the heterologous strains has been examined. In the bottom two panels of Figure 20 are
15 electron micrographs demonstrating the reactivity of Mab AD6 with surface-accessible epitopes on nontypable Haemophilus strains 5 and 15. As can be seen, abundant colloidal-gold particles are evident on the surfaces of each of these strains, confirming their surface
20 expression of the AD6 epitope. Although limited in scope, these data suggest that the AD6 epitope may be a common surface-accessible epitope on the high molecular weight adhesion proteins of most nontypable Haemophilus influenzae which express HMW1/HMW2-like proteins.

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SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides high molecular weight proteins of non-typeable
30 Haemophilus, genes coding for the same and vaccines incorporating such proteins. Modifications are possible within the scope of this invention.

TABLE 1: Effect of mutation of high molecular weight proteins on adherence to Chang epithelial cells by nontypable *H. influenzae*.

<u>Strain</u>	<u>ADHERENCE % *</u>	
	<u>% Inoculation</u>	<u>Relative to wild Type†</u>
Strain 12 derivatives wild type	87.76 ± 5.9	100.0 ± 6.7
HMW1 ⁻ mutant	6.0 ± 0.9	6.8 ± 1.0
HMW2 ⁻ mutant	89.9 ± 10.8	102.5 ± 12.3
HMW1 ⁻ /HMW2 ⁻ mutant	2.0 ± 0.3	2.3 ± 0.3
Strain 5 derivatives wild type	78.7 ± 3.2	100.0 ± 4.1
HMW1-like mutant	15.7 ± 2.6	19.9 ± 3.3
HMW2-like mutant	103.7 ± 14.0	131.7 ± 17.8
double mutant	3.5 ± 0.6	4.4 ± 0.8

* Numbers represent mean (± standard error of the mean) of measurements in triplicate or quadruplicate from representative experiments.

† Adherence values for strain 12 derivatives are relative to strain 12 wild type; values for strain 5 derivatives are relative to strain 5 wild type.

TABLE 2: Adherence by *E. coli* DH5 α and HB101 harboring *hmw1* or *hmw2* gene clusters.

Strain*	Adherence relative to <i>H. influenzae</i> strain 12†
DH5 α (pT7-7)	0.7 \pm 0.02
DH5 α (pHMW1-14)	114.2 \pm 15.9
DH5 α (pHMW2-21)	14.0 \pm 3.7
HB101 (pT7-7)	1.2 \pm 0.5
HB101 (pHMW1-14)	93.6 \pm 15.8
HB101 (pHMW2-21)	3.6 \pm 0.9

* The plasmid pHMW1-14 contains the *hmw1* gene cluster, while pHMW2-21 contains the *hmw2* gene cluster; pT7-7 is the cloning vector used in these constructs.

† Numbers represent the mean (\pm standard error of the mean) of measurements made in triplicate from representative experiments.

TABLE 3: Protective ability of HMW protein against non-typeable *H. influenzae* challenge in chinchilla model

Group (#)	Antigens	Total Animals	Number of Animals Showed Positive Ear Infection		
			Tympano- gram	Otosco- pic Examin- ation	cfu of Bacteria /10 μ L
1	HMW	5	0	0	0
2	None	5	5	5	850- 3200 (4/5)
3	Convalescent	4	0	0	0

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Barenkamp, Stephen J
- (ii) TITLE OF INVENTION: High Molecular Weight Surface Proteins of Non-Typeable Haemophilus
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
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 - (F) ZIP: 22202-0286
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
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 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
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 - (A) APPLICATION NUMBER: US PCT/US93/02166
 - (B) FILING DATE: 16-MAR-1993
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5116 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1536 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Ala Thr Met Gln Val Asp Gly Asn Lys Thr Ile Ile Arg Asn Ser Val
      85             90             95
Asp Ala Ile Ile Asn Trp Lys Gln Phe Asn Ile Asp Gln Asn Glu Met
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      115            120            125

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67

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 Ile Ile Asn Thr Asn Gly Phe Thr Ala Ser Thr Leu Asp Ile Ser Asn
 165 170 175
 Glu Asn Ile Lys Ala Arg Asn Phe Thr Phe Glu Gln Thr Lys Asp Lys
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 Ala Leu Ala Glu Ile Val Asn His Gly Leu Ile Thr Val Gly Lys Asp
 195 200 205
 Gly Ser Val Asn Leu Ile Gly Gly Lys Val Lys Asn Glu Gly Val Ile
 210 215 220
 Ser Val Asn Gly Gly Ser Ile Ser Leu Leu Ala Gly Gln Lys Ile Thr
 225 230 235 240
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 245 250 255
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 260 265 270
 Ile Asn Val Arg Ala Ala Thr Ile Arg Asn Gln Gly Lys Leu Ser Ala
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 Phe Val Glu Thr Ser Gly His Asp Leu Phe Ile Lys Asp Asn Ala Ile
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68

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 Thr Asn Asn Ala Asn Leu Thr Ile Lys Thr Lys Glu Leu Lys Leu Thr
 1025 1030 1035 1040
 Gln Asp Leu Asn Ile Ser Gly Phe Asn Lys Ala Glu Ile Thr Ala Lys
 1045 1050 1055
 Asp Gly Ser Asp Leu Thr Ile Gly Asn Thr Asn Ser Ala Asp Gly Thr
 1060 1065 1070
 Asn Ala Lys Lys Val Thr Phe Asn Gln Val Lys Asp Ser Lys Ile Ser
 1075 1080 1085
 Ala Asp Gly His Lys Val Thr Leu His Ser Lys Val Glu Thr Ser Gly
 1090 1095 1100
 Ser Asn Asn Asn Thr Glu Asp Ser Ser Asp Asn Asn Ala Gly Leu Thr
 1105 1110 1115 1120
 Ile Asp Ala Lys Asn Val Thr Val Asn Asn Asn Ile Thr Ser His Lys
 1125 1130 1135
 Ala Val Ser Ile Ser Ala Thr Ser Gly Glu Ile Thr Thr Lys Thr Gly
 1140 1145 1150
 Thr Thr Ile Asn Ala Thr Thr Gly Asn Val Glu Ile Thr Ala Gln Thr
 1155 1160 1165
 Gly Ser Ile Leu Gly Gly Ile Glu Ser Ser Ser Gly Ser Val Thr Leu
 1170 1175 1180

Thr Ala Thr Glu Gly Ala Leu Ala Val Ser Asn Ile Ser Gly Asn Thr
 1185 1190 1195 1200
 Val Thr Val Thr Ala Asn Ser Gly Ala Leu Thr Thr Leu Ala Gly Ser
 1205 1210 1215
 Thr Ile Lys Gly Thr Glu Ser Val Thr Thr Ser Ser Gln Ser Gly Asp
 1220 1225 1230
 Ile Gly Gly Thr Ile Ser Gly Gly Thr Val Glu Val Lys Ala Thr Glu
 1235 1240 1245
 Ser Leu Thr Thr Gln Ser Asn Ser Lys Ile Lys Ala Thr Thr Gly Glu
 1250 1255 1260
 Ala Asn Val Thr Ser Ala Thr Gly Thr Ile Gly Gly Thr Ile Ser Gly
 1265 1270 1275 1280
 Asn Thr Val Asn Val Thr Ala Asn Ala Gly Asp Leu Thr Val Gly Asn
 1285 1290 1295
 Gly Ala Glu Ile Asn Ala Thr Glu Gly Ala Ala Thr Leu Thr Thr Ser
 1300 1305 1310
 Ser Gly Lys Leu Thr Thr Glu Ala Ser Ser His Ile Thr Ser Ala Lys
 1315 1320 1325
 Gly Gln Val Asn Leu Ser Ala Gln Asp Gly Ser Val Ala Gly Ser Ile
 1330 1335 1340
 Asn Ala Ala Asn Val Thr Leu Asn Thr Thr Gly Thr Leu Thr Thr Val
 1345 1350 1355 1360
 Lys Gly Ser Asn Ile Asn Ala Thr Ser Gly Thr Leu Val Ile Asn Ala
 1365 1370 1375
 Lys Asp Ala Glu Leu Asn Gly Ala Ala Leu Gly Asn His Thr Val Val
 1380 1385 1390
 Asn Ala Thr Asn Ala Asn Gly Ser Gly Ser Val Ile Ala Thr Thr Ser
 1395 1400 1405
 Ser Arg Val Asn Ile Thr Gly Asp Leu Ile Thr Ile Asn Gly Leu Asn
 1410 1415 1420
 Ile Ile Ser Lys Asn Gly Ile Asn Thr Val Leu Leu Lys Gly Val Lys
 1425 1430 1435 1440
 Ile Asp Val Lys Tyr Ile Gln Pro Gly Ile Ala Ser Val Asp Glu Val
 1445 1450 1455
 Ile Glu Ala Lys Arg Ile Leu Glu Lys Val Lys Asp Leu Ser Asp Glu
 1460 1465 1470
 Glu Arg Glu Ala Leu Ala Lys Leu Gly Val Ser Ala Val Arg Phe Ile
 1475 1480 1485
 Glu Pro Asn Asn Thr Ile Thr Val Asp Thr Gln Asn Glu Phe Ala Thr
 1490 1495 1500
 Arg Pro Leu Ser Arg Ile Val Ile Ser Glu Gly Arg Ala Cys Phe Ser
 1505 1510 1515 1520
 Asn Ser Asp Gly Ala Thr Val Cys Val Asn Ile Ala Asp Asn Gly Arg
 1525 1530 1535

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4937 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TAAATATACA AGATAATAAA AATAAATCAA GATTTTTGTG ATGACAAACA ACAATTACAA	60
CACCTTTTTT GCAGTCTATA TGCAAATATT TTAACAAAAAT AGTATAAATC CGCCATATAA	120
AATGGTATAA TCTTTCATCT TTCATCTTTA ATCTTTCATC TTTCATCTTT CATCTTTCAT	180
CTTTCATCTT TCATCTTTCA TCTTTCATCT TTCATCTTTC ATCTTTCATC TTTCATCTTT	240
CACATGAAAT GATGAACCGA GGAAGGGGAG GGAGGGGCAA GAATGAAGAG GGAGCTGAAC	300
GAACGCAAAT GATAAAGTAA TTTAATTGTT CAACTAACCT TAGGAGAAAA TATGAACAAG	360
ATATATCGTC TCAAATTCAG CAAACGCCTG AATGCTTTGG TTGCTGTGTC TGAATTGGCA	420
CGGGGTTGTG ACCATTCCAC AGAAAAAGGC TTCCGCTATG TTACTATCTT TAGGTGTAAC	480
CACTTAGCGT TAAAGCCACT TTCCGCTATG TTACTATCTT TAGGTGTAAC ATCTATTCCA	540
CAATCTGTTT TAGCAAGCGG CTTACAAGGA ATGGATGTAG TACACGGCAC AGCCACTATG	600
CAAGTAGATG GTAATAAAAC CATTATCCGC AACAGTGTG ACGCTATCAT TAATTGGAAA	660
CAATTTAACA TCGACCAAAA TGAAATGGTG CAGTTTTTAC AAGAAAACAA CAACTCCGCC	720
GTATTCAACC GTGTTACATC TAACCAAATC TCCCAATTAA AAGGGATTTT AGATTCTAAC	780
GGACAAGTCT TTTTAATCAA CCCAAATGGT ATCACAATAG GTAAAGACGC AATTATTAAC	840
ACTAATGGCT TTACGGCTTC TACGCTAGAC ATTTCTAACG AAAACATCAA GCGCGTAAT	900
TTCACCTTCG AGCAAACCAA AGATAAAGCG CTCGCTGAAA TTGTGAATCA CGGTTTAATT	960
ACTGTCGGTA AAGACGGCAG TGTAATCTT ATTGGTGGCA AAGTGAAAAA CGAGGGTGTG	1020
ATTAGCGTAA ATGGTGGCAG CATTTCTTTA CTCGCAGGCG AAAAATCAC CATCAGCGAT	1080
ATAATAAACC CAACCATTAC TTACAGCATT GCCGCGCCTG AAAATGAAGC GGTCAATCTG	1140
GGCGATATTT TTGCCAAAGG CGGTAACATT AATGTCCGTG CTGCCACTAT TCGAAACCAA	1200
GGTAAACTTT CTGCTGATTC TGTAAGCAAA GATAAAAGCG GCAATATTGT TCTTTCGCC	1260
AAAGAGGGTG AAGCGGAAAT TGGCGGTGTA ATTTCCGCTC AAAATCAGCA AGCTAAAGGC	1320
GGCAAGCTGA TGATTACAGG CGATAAAGTC ACATTAAAAA CAGGTGCAGT TATCGACCTT	1380
TCAGGTAAAG AAGGGGGAGA AACTTACCTT GCGCGGTGACG AGCGCGGCGA AGGTAAAAAC	1440
GGCATTCAAT TAGCAAAGAA AACCTCTTTA GAAAAAGGCT CAACCATCAA TGTATCAGGC	1500
AAAGAAAAAG GCGGACGCGC TATTGTGTGG GCGGATATTG CGTTAATTGA CGGCAATATT	1560
AACGCTCAAG GTAGTGGTGA TATCGCTAAA ACCGGTGGTT TTGTGGAGAC ATCGGGGCAT	1620
TATTTATCCA TTGACAGCAA TGCAATTGTT AAAACAAAAG AGTGGTTGCT AGACCCTGAT	1680

GATGTAACAA	TTGAAGCCGA	AGACCCCCTT	CGCAATAATA	CCGGTATAAA	TGATGAATTC	1740
CCAACAGGCA	CCGGTGAAGC	AAGCGACCCT	AAAAAAAATA	GCGAACTCAA	AACAACGCTA	1800
ACCAATACAA	CTATTTCAAA	TTATCTGAAA	AACGCCTGGA	CAATGAATAT	AACGGCATCA	1860
AGAAAACCTA	CCGTTAATAG	CTCAATCAAC	ATCGGAAGCA	ACTCCCACCT	AATTCTCCAT	1920
AGTAAAGGTC	AGCGTGGCGG	AGGCGTTTCA	ATTGATGGAG	ATATTACTTC	TAAAGGCGGA	1980
AATTTAACCA	TTTATTCTGG	CGGATGGGTT	GATGTTTATA	AAAATATTAC	GCTTGATCAG	2040
GGTTTTTTAA	ATATTACCGC	CGCTTCCGTA	GCTTTTGAAG	GTGGAAATAA	CAAAGCACGC	2100
GACGCGGCAA	ATGCTAAAAT	TGTCGCCCAG	GGCACTGTAA	CCATTACAGG	AGAGGGAAAA	2160
GATTTTCAGG	CTAACAACGT	ATCTTTAAAC	GGAACGGGTA	AAGGTCTGAA	TATCATTTCA	2220
TCAGTGAATA	ATTTAACCCA	CAATCTTAGT	GGCACAATTA	ACATATCTGG	GAATATAACA	2280
ATTAACCAAA	CTACGAGAAA	GAACACCTCG	TATTGGCAAA	CCAGCCATGA	TTCGCACTGG	2340
AACGTCAGTG	CTCTTAATCT	AGAGACAGGC	GCAAATTTTA	CCTTTATTAA	ATACATTTCA	2400
AGCAATAGCA	AAGGCTTAAC	AACACAGTAT	AGAAGCTCTG	CAGGGGTGAA	TTTTAACGGC	2460
GTAAATGGCA	ACATGTCATT	CAATCTCAAA	GAAGGAGCGA	AAGTTAATTT	CAAATTAATA	2520
CCAAACGAGA	ACATGAACAC	AAGCAAACCT	TTACCAATTC	GGTTTTTAGC	CAATATCACA	2580
GCCACTGGTG	GGGGCTCTGT	TTTTTTTGAT	ATATATGCCA	ACCATTCTGG	CAGAGGGGCT	2640
GAGTTAAAAA	TGAGTGAAAT	TAATATCTCT	AACGGCGCTA	ATTTTACCTT	AAATTCCCAT	2700
GTTTCGGGCG	ATGACGCTTT	TAAAATCAAC	AAAGACTTAA	CCATAAATGC	AACCAATTCA	2760
AATTTTCAGC	TCAGACAGAC	GAAAGATGAT	TTTTATGACG	GGTACGCACG	CAATGCCATC	2820
AATTC AACCT	ACAACATATC	CATTCTGGGC	GGTAATGTCA	CCCTTGGTGG	ACAAAACCTCA	2880
AGCAGCAGCA	TTACGGGGAA	TATTACTATC	GAGAAAGCAG	CAAATGTTAC	GCTAGAAGCC	2940
AATAACGCCC	CTAATCAGCA	AAACATAAGG	GATAGAGTTA	TAAACTTGG	CAGCTTGCTC	3000
GTTAATGGGA	GTTTAAGTTT	AACTGGCGAA	AATGCAGATA	TTAAAGGCAA	TCTCACTATT	3060
TCAGAAAGCG	CCACTTTTAA	AGGAAAAGCT	AGAGATACCC	TAAATATCAC	CGGCAATTTT	3120
ACCAATAATG	GCACTGCCGA	AATTAATATA	ACACAAGGAG	TGGTAAACT	TGGCAATGTT	3180
ACCAATGATG	GTGATTTAAA	CATTACCACT	CACGCTAAAC	GCAACCAAAG	AAGCATCATC	3240
GGCGGAGATA	TAATCAACAA	AAAAGGAAGC	TTAAATATTA	CAGACAGTAA	TAATGATGCT	3300
GAAATCCAAA	TTGGCGGCAA	TATCTCGCAA	AAAGAAGGCA	ACCTCACGAT	TTCTTCCGAT	3360
AAAATTAATA	TCACCAAACA	GATAACAATC	AAAAAGGGTA	TTGATGGAGA	GGACTCTAGT	3420
TCAGATGCGA	CAAGTAATGC	CAACCTAACT	ATTAAAACCA	AAGAATTGAA	ATTGACAGAA	3480
GACCTAAGTA	TTTCAGGTTT	CAATAAAGCA	GAGATTACAG	CCAAAGATGG	TAGAGATTTA	3540
ACTATTGGCA	ACAGTAATGA	CGGTAACAGC	GGTGCCGAAG	CCAAAACAGT	AACCTTTAAC	3600
AATGTTAAAG	ATTCAAAAAT	CTCTGCTGAC	GGTCACAATG	TGACACTAAA	TAGCAAAGTG	3660
AAAACATCTA	GCAGCAATGG	CGGACGTGAA	AGCAATAGCG	ACAACGATAC	CGGCTTAACT	3720

73

ATTACTGCAA AAAATGTAGA AGTAAACAAA GATATTACTT CTCTCAAAAC AGTAAATATC 3780
 ACCGCGTCGG AAAAGGTTAC CACCACAGCA GGCTCGACCA TTAACGCAAC AAATGGCAAA 3840
 GCAAGTATTA CAACCAAAAC AGGTGATATC AGCGGTACGA TTTCCGGTAA CACGGTAAGT 3900
 GTTAGCGCGA CTGGTGATTT AACCCTAAA TCCGGCTCAA AAATTGAAGC GAAATCGGGT 3960
 GAGGCTAATG TAACAAGTGC AACAGGTACA ATTGGCGGTA CAATTTCCGG TAATACGGTA 4020
 AATGTTACGG CAAACGCTGG CGATTTAACA GTTGGGAATG GCGCAGAAAT TAATGCGACA 4080
 GAAGGAGCTG CAACCTTAAC CGCAACAGGG AATACCTTGA CTACTGAAGC CGGTTCTAGC 4140
 ATCACTTCAA CTAAGGTCA GGTAGACCTC TTGGCTCAGA ATGGTAGCAT CGCAGGAAGC 4200
 ATTAATGCTG CTAATGTGAC ATTAAATACT ACAGGCACCT TAACCACCGT GGCAGGCTCG 4260
 GATATTAAAG CAACCAGCGG CACCTTGGTT ATTAACGCAA AAGATGCTAA GCTAAATGGT 4320
 GATGCATCAG GTGATAGTAC AGAAGTGAAT GCAGTCAACG CAAGCGGCTC TGGTAGTGTG 4380
 ACTGCGGCAA CCTCAAGCAG TGTGAATATC ACTGGGGATT TAAACACAGT AAATGGGTTA 4440
 AATATCATTT CGAAAGATGG TAGAAACACT GTGCGCTTAA GAGGCAAGGA AATTGAGGTG 4500
 AAATATATCC AGCCAGGTGT AGCAAGTGTA GAAGAAGTAA TTGAAGCGAA ACGCGTCCTT 4560
 GAAAAAGTAA AAGATTTATC TGATGAAGAA AGAGAAACAT TAGCTAAACT TGGTGTAAGT 4620
 GCTGTACGTT TTGTTGAGCC AAATAATACA ATTACAGTCA ATACACAAAA TGAATTTACA 4680
 ACCAGACCGT CAAGTCAAGT GATAATTTCT GAAGGTAAGG CGTGTCTTCTC AAGTGGTAAT 4740
 GCGCACGAG TATGTACCAA TGTGCTGAC GATGGACAGC CGTAGTCAGT AATTGACAAG 4800
 GTAGATTTCA TCCTGCAATG AAGTCATTTT ATTTTCGTAT TATTTACTGT GTGGGTTAAA 4860
 GTTCAGTACG GGCTTTACCC ATCTTGTAAG AAATTACGGA GAATACAATA AAGTATTTTT 4920
 AACAGGTTAT TATTATG 4937

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1477 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Lys Ile Tyr Arg Leu Lys Phe Ser Lys Arg Leu Asn Ala Leu
 1 5 10 15
 Val Ala Val Ser Glu Leu Ala Arg Gly Cys Asp His Ser Thr Glu Lys
 20 25 30
 Gly Ser Glu Lys Pro Ala Arg Met Lys Val Arg His Leu Ala Leu Lys
 35 40 45
 Pro Leu Ser Ala Met Leu Leu Ser Leu Gly Val Thr Ser Ile Pro Gln
 50 55 60

74

Ser Val Leu Ala Ser Gly Leu Gln Gly Met Asp Val Val His Gly Thr
 65 70 75 80
 Ala Thr Met Gln Val Asp Gly Asn Lys Thr Ile Ile Arg Asn Ser Val
 85 90 95
 Asp Ala Ile Ile Asn Trp Lys Gln Phe Asn Ile Asp Gln Asn Glu Met
 100 105 110
 Val Gln Phe Leu Gln Glu Asn Asn Asn Ser Ala Val Phe Asn Arg Val
 115 120 125
 Thr Ser Asn Gln Ile Ser Gln Leu Lys Gly Ile Leu Asp Ser Asn Gly
 130 135 140
 Gln Val Phe Leu Ile Asn Pro Asn Gly Ile Thr Ile Gly Lys Asp Ala
 145 150 155 160
 Ile Ile Asn Thr Asn Gly Phe Thr Ala Ser Thr Leu Asp Ile Ser Asn
 165 170 175
 Glu Asn Ile Lys Ala Arg Asn Phe Thr Phe Glu Gln Thr Lys Asp Lys
 180 185 190
 Ala Leu Ala Glu Ile Val Asn His Gly Leu Ile Thr Val Gly Lys Asp
 195 200 205
 Gly Ser Val Asn Leu Ile Gly Gly Lys Val Lys Asn Glu Gly Val Ile
 210 215 220
 Ser Val Asn Gly Gly Ser Ile Ser Leu Leu Ala Gly Gln Lys Ile Thr
 225 230 235 240
 Ile Ser Asp Ile Ile Asn Pro Thr Ile Thr Tyr Ser Ile Ala Ala Pro
 245 250 255
 Glu Asn Glu Ala Val Asn Leu Gly Asp Ile Phe Ala Lys Gly Gly Asn
 260 265 270
 Ile Asn Val Arg Ala Ala Thr Ile Arg Asn Gln Gly Lys Leu Ser Ala
 275 280 285
 Asp Ser Val Ser Lys Asp Lys Ser Gly Asn Ile Val Leu Ser Ala Lys
 290 295 300
 Glu Gly Glu Ala Glu Ile Gly Gly Val Ile Ser Ala Gln Asn Gln Gln
 305 310 315 320
 Ala Lys Gly Gly Lys Leu Met Ile Thr Gly Asp Lys Val Thr Leu Lys
 325 330 335
 Thr Gly Ala Val Ile Asp Leu Ser Gly Lys Glu Gly Gly Glu Thr Tyr
 340 345 350
 Leu Gly Gly Asp Glu Arg Gly Glu Gly Lys Asn Gly Ile Gln Leu Ala
 355 360 365
 Lys Lys Thr Ser Leu Glu Lys Gly Ser Thr Ile Asn Val Ser Gly Lys
 370 375 380
 Glu Lys Gly Gly Phe Ala Ile Val Trp Gly Asp Ile Ala Leu Ile Asp
 385 390 395 400
 Gly Asn Ile Asn Ala Gln Gly Ser Gly Asp Ile Ala Lys Thr Gly Gly
 405 410 415

Phe Val Glu Thr Ser Gly His Asp Leu Phe Ile Lys Asp Asn Ala Ile
 420 425 430
 Val Asp Ala Lys Glu Trp Leu Leu Asp Phe Asp Asn Val Ser Ile Asn
 435 440 445
 Ala Glu Asp Pro Leu Phe Asn Asn Thr Gly Ile Asn Asp Glu Phe Pro
 450 455 460
 Thr Gly Thr Gly Glu Ala Ser Asp Pro Lys Lys Asn Ser Glu Leu Lys
 465 470 475 480
 Thr Thr Leu Thr Asn Thr Thr Ile Ser Asn Tyr Leu Lys Asn Ala Trp
 485 490 495
 Thr Met Asn Ile Thr Ala Ser Arg Lys Leu Thr Val Asn Ser Ser Ile
 500 505 510
 Asn Ile Gly Ser Asn Ser His Leu Ile Leu His Ser Lys Gly Gln Arg
 515 520 525
 Gly Gly Gly Val Gln Ile Asp Gly Asp Ile Thr Ser Lys Gly Gly Asn
 530 535 540
 Leu Thr Ile Tyr Ser Gly Gly Trp Val Asp Val His Lys Asn Ile Thr
 545 550 555 560
 Leu Asp Gln Gly Phe Leu Asn Ile Thr Ala Ala Ser Val Ala Phe Glu
 565 570 575
 Gly Gly Asn Asn Lys Ala Arg Asp Ala Ala Asn Ala Lys Ile Val Ala
 580 585 590
 Gln Gly Thr Val Thr Ile Thr Gly Glu Gly Lys Asp Phe Arg Ala Asn
 595 600 605
 Asn Val Ser Leu Asn Gly Thr Gly Lys Gly Leu Asn Ile Ile Ser Ser
 610 615 620
 Val Asn Asn Leu Thr His Asn Leu Ser Gly Thr Ile Asn Ile Ser Gly
 625 630 635 640
 Asn Ile Thr Ile Asn Gln Thr Thr Arg Lys Asn Thr Ser Tyr Trp Gln
 645 650 655
 Thr Ser His Asp Ser His Trp Asn Val Ser Ala Leu Asn Leu Glu Thr
 660 665 670
 Gly Ala Asn Phe Thr Phe Ile Lys Tyr Ile Ser Ser Asn Ser Lys Gly
 675 680 685
 Leu Thr Thr Gln Tyr Arg Ser Ser Ala Gly Val Asn Phe Asn Gly Val
 690 695 700
 Asn Gly Asn Met Ser Phe Asn Leu Lys Glu Gly Ala Lys Val Asn Phe
 705 710 715 720
 Lys Leu Lys Pro Asn Glu Asn Met Asn Thr Ser Lys Pro Leu Pro Ile
 725 730 735
 Arg Phe Leu Ala Asn Ile Thr Ala Thr Gly Gly Gly Ser Val Phe Phe
 740 745 750
 Asp Ile Tyr Ala Asn His Ser Gly Arg Gly Ala Glu Leu Lys Met Ser
 755 760 765

76:

Glu Ile Asn Ile Ser Asn Gly Ala Asn Phe Thr Leu Asn Ser His Val
 770 775 780
 Arg Gly Asp Asp Ala Phe Lys Ile Asn Lys Asp Leu Thr Ile Asn Ala
 785 790 795 800
 Thr Asn Ser Asn Phe Ser Leu Arg Gln Thr Lys Asp Asp Phe Tyr Asp
 805 810 815
 Gly Tyr Ala Arg Asn Ala Ile Asn Ser Thr Tyr Asn Ile Ser Ile Leu
 820 825 830
 Gly Gly Asn Val Thr Leu Gly Gly Gln Asn Ser Ser Ser Ser Ile Thr
 835 840 845
 Gly Asn Ile Thr Ile Glu Lys Ala Ala Asn Val Thr Leu Glu Ala Asn
 850 855 860
 Asn Ala Pro Asn Gln Gln Asn Ile Arg Asp Arg Val Ile Lys Leu Gly
 865 870 875 880
 Ser Leu Leu Val Asn Gly Ser Leu Ser Leu Thr Gly Glu Asn Ala Asp
 885 890 895
 Ile Lys Gly Asn Leu Thr Ile Ser Glu Ser Ala Thr Phe Lys Gly Lys
 900 905 910
 Thr Arg Asp Thr Leu Asn Ile Thr Gly Asn Phe Thr Asn Asn Gly Thr
 915 920 925
 Ala Glu Ile Asn Ile Thr Gln Gly Val Val Lys Leu Gly Asn Val Thr
 930 935 940
 Asn Asp Gly Asp Leu Asn Ile Thr Thr His Ala Lys Arg Asn Gln Arg
 945 950 955 960
 Ser Ile Ile Gly Gly Asp Ile Ile Asn Lys Lys Gly Ser Leu Asn Ile
 965 970 975
 Thr Asp Ser Asn Asn Asp Ala Glu Ile Gln Ile Gly Gly Asn Ile Ser
 980 985 990
 Gln Lys Glu Gly Asn Leu Thr Ile Ser Ser Asp Lys Ile Asn Ile Thr
 995 1000 1005
 Lys Gln Ile Thr Ile Lys Lys Gly Ile Asp Gly Glu Asp Ser Ser Ser
 1010 1015 1020
 Asp Ala Thr Ser Asn Ala Asn Leu Thr Ile Lys Thr Lys Glu Leu Lys
 1025 1030 1035 1040
 Leu Thr Glu Asp Leu Ser Ile Ser Gly Phe Asn Lys Ala Glu Ile Thr
 1045 1050 1055
 Ala Lys Asp Gly Arg Asp Leu Thr Ile Gly Asn Ser Asn Asp Gly Asn
 1060 1065 1070
 Ser Gly Ala Glu Ala Lys Thr Val Thr Phe Asn Asn Val Lys Asp Ser
 1075 1080 1085
 Lys Ile Ser Ala Asp Gly His Asn Val Thr Leu Asn Ser Lys Val Lys
 1090 1095 1100
 Thr Ser Ser Ser Asn Gly Gly Arg Glu Ser Asn Ser Asp Asn Asp Thr
 1105 1110 1115 1120

Gly Leu Thr Ile Thr Ala Lys Asn Val Glu Val Asn Lys Asp Ile Thr
 1125 1130 1135
 Ser Leu Lys Thr Val Asn Ile Thr Ala Ser Glu Lys Val Thr Thr Thr
 1140 1145 1150
 Ala Gly Ser Thr Ile Asn Ala Thr Asn Gly Lys Ala Ser Ile Thr Thr
 1155 1160 1165
 Lys Thr Gly Asp Ile Ser Gly Thr Ile Ser Gly Asn Thr Val Ser Val
 1170 1175 1180
 Ser Ala Thr Val Asp Leu Thr Thr Lys Ser Gly Ser Lys Ile Glu Ala
 1185 1190 1195 1200
 Lys Ser Gly Glu Ala Asn Val Thr Ser Ala Thr Gly Thr Ile Gly Gly
 1205 1210 1215
 Thr Ile Ser Gly Asn Thr Val Asn Val Thr Ala Asn Ala Gly Asp Leu
 1220 1225 1230
 Thr Val Gly Asn Gly Ala Glu Ile Asn Ala Thr Glu Gly Ala Ala Thr
 1235 1240 1245
 Leu Thr Ala Thr Gly Asn Thr Leu Thr Thr Glu Ala Gly Ser Ser Ile
 1250 1255 1260
 Thr Ser Thr Lys Gly Gln Val Asp Leu Leu Ala Gln Asn Gly Ser Ile
 1265 1270 1275 1280
 Ala Gly Ser Ile Asn Ala Ala Asn Val Thr Leu Asn Thr Thr Gly Thr
 1285 1290 1295
 Leu Thr Thr Val Ala Gly Ser Asp Ile Lys Ala Thr Ser Gly Thr Leu
 1300 1305 1310
 Val Ile Asn Ala Lys Asp Ala Lys Leu Asn Gly Asp Ala Ser Gly Asp
 1315 1320 1325
 Ser Thr Glu Val Asn Ala Val Asn Ala Ser Gly Ser Gly Ser Val Thr
 1330 1335 1340
 Ala Ala Thr Ser Ser Ser Val Asn Ile Thr Gly Asp Leu Asn Thr Val
 1345 1350 1355 1360
 Asn Gly Leu Asn Ile Ile Ser Lys Asp Gly Arg Asn Thr Val Arg Leu
 1365 1370 1375
 Arg Gly Lys Glu Ile Glu Val Lys Tyr Ile Gln Pro Gly Val Ala Ser
 1380 1385 1390
 Val Glu Glu Val Ile Glu Ala Lys Arg Val Leu Glu Lys Val Lys Asp
 1395 1400 1405
 Leu Ser Asp Glu Glu Arg Glu Thr Leu Ala Lys Leu Gly Val Ser Ala
 1410 1415 1420
 Val Arg Phe Val Glu Pro Asn Asn Thr Ile Thr Val Asn Thr Gln Asn
 1425 1430 1435 1440
 Glu Phe Thr Thr Arg Pro Ser Ser Gln Val Ile Ile Ser Glu Gly Lys
 1445 1450 1455
 Ala Cys Phe Ser Ser Gly Asn Gly Ala Arg Val Cys Thr Asn Val Ala
 1460 1465 1470

Asp Asp Gly Gln Pro
1475

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9171 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACAGCGTTCT	CTTAATACTA	GTACAAACCC	ACAATAAAAT	ATGACAAACA	ACAATTACAA	60
CACCTTTTTT	GCAGTCTATA	TGCAAATATT	TTAAAAATA	GTATAAATCC	GCCATATAAA	120
ATGGTATAAT	CTTTCATCTT	TCATCTTTCA	TCTTTCATCT	TTCATCTTTC	ATCTTTCATC	180
TTTCATCTTT	CATCTTTCAT	CTTTCATCTT	TCATCTTTCA	TCTTTCATCT	TTCATCTTTC	240
ACATGAAATG	ATGAACCGAG	GGAAGGGAGG	GAGGGGCAAG	AATGAAGAGG	GAGCTGAACG	300
AACGCAAATG	ATAAAGTAAT	TTAATTGTTT	AACCTAACCTT	AGGAGAAAAT	ATGAACAAGA	360
TATATCGTCT	CAAATTCAGC	AAACGCCTGA	ATGCTTTGGT	TGCTGTGTCT	GAATTGGCAC	420
GGGGTTGTGA	CCATTCCACA	GAAAAAGGCA	GCGAAAAACC	TGCTCGCATG	AAAGTGCGTC	480
ACTTAGCGTT	AAAGCCACTT	TCCGCTATGT	TACTATCTTT	AGGTGTAACA	TCTATTCCAC	540
AATCTGTTTT	AGCAAGCGGC	TTACAAGGAA	TGGATGTAGT	ACACGGCACA	GCCACTATGC	600
AAGTAGATGG	TAATAAAACC	ATTATCCGCA	ACAGTGTGTA	CGCTATCATT	AATTGGAAAC	660
AATTTAACAT	CGACCAAAT	GAAATGGTGC	AGTTTTTACA	AGAAAACAAC	AACTCCGCCG	720
TATTCAACCG	TGTTACATCT	AACCAAATCT	CCCAATTAAA	AGGGATTTTA	GATTCTAACG	780
GACAAGTCTT	TTTAATCAAC	CCAAATGGTA	TCACAATAGG	TAAAGACGCA	ATTATTAACA	840
CTAATGGCTT	TACGGCTTCT	ACGCTAGACA	TTTCTAACGA	AAACATCAAG	GCGCGTAATT	900
TCACCTTCGA	GCAAACCAA	GATAAAGCGC	TCGCTGAAAT	TGTGAATCAC	GGTTTAATTA	960
CTGTCGGTAA	AGACGGCAGT	GTAAATCTTA	TTGGTGGCAA	AGTGAAAAAC	GAGGGTGTGA	1020
TTAGCGTAAA	TGGTGGCAGC	ATTTCTTTAC	TCGCAGGGCA	AAAAATCACC	ATCAGCGATA	1080
TAATAAACCC	AACCATTACT	TACAGCATTG	CCGCGCCTGA	AAATGAAGCG	GTCAATCTGG	1140
GCGATATTTT	TGCCAAAGGC	GGTAACATTA	ATGTCCGTGC	TGCCACTATT	CGAAACCAAG	1200
CTTTCGCCCA	AAGAGGGTGA	AGCGGAAATT	GGCGGTGTAA	TTTCCGCTCA	AAATCAGCAA	1260
GCTAAAGGCG	GCAAGCTGAT	GATTACAGGC	GATAAAGTCA	CATTAAAAAC	AGGTGCAGTT	1320
ATCGACCTTT	CAGGTAAAGA	AGGGGGAGAA	ACTTACCTTG	GCGGTGACGA	GCGCGGCGAA	1380
GGTAAAAACG	GCATTCAATT	AGCAAAGAAA	ACCTCTTTAG	AAAAAGGCTC	AACCATCAAT	1440
GTATCAGGCA	AAGAAAAAGG	CGGACGCGCT	ATTGTGTGGG	GCGATATTGC	GTTAATTGAC	1500

GGCAATATTA	ACGCTCAAGG	TAGTGGTGAT	ATCGCTAAAA	CCGGTGGTTT	TGTGGAGACG	1560
TCCGGGCGATG	ATTTATTCAT	CAAAGACAAT	GCAATTGTTG	ACGCCAAAGA	GTGGTTGTTA	1620
GACCCGGATA	ATGTATCTAT	TAATGCAGAA	ACAGCAGGAC	GCAGCAATAC	TTCAGAAGAC	1680
GATGAATACA	CGGGATCCCG	GAATAGTGCC	AGCACCCCAA	AACGAAACAA	AGAAAAGACA	1740
ACATTAAACAA	ACACAACTCT	TGAGAGTATA	CTAAAAAAG	GTACCTTTGT	TAACATCACT	1800
GCTAATCAAC	GCATCTATGT	CAATAGCTCC	ATTAATTTAT	CCAATGGCAG	CTTAACCTTT	1860
TGGAGTGAGG	GTCGGAGCGG	TGGCGGCGTT	GAGATTAAAC	ACGATATTAC	CACCGGTGAT	1920
GATACCAGAG	GTGCAAACTT	AACAATTTAC	TCAGGCGGCT	GGGTTGATGT	TCATAAAAAAT	1980
ATCTCACTCG	GGGCGCAAGG	TAACATAAAC	ATTACAGCTA	AACAAGATAT	CGCCTTTGAG	2040
AAAGGAAGCA	ACCAAGTCAT	TACAGGTCAA	GGGACTATTA	CCTCAGGCAA	TCAAAAAGGT	2100
TTTAGATTTA	ATAATGTCTC	TCTAAACGGC	ACTGGCAGCG	GACTGCAATT	CACCACTAAA	2160
AGAACCAATA	AATACGCTAT	CACAAATAAA	TTTGAAGGGA	CTTTAAATAT	TTCAGGGAAA	2220
GTGAACATCT	CAATGGTTTT	ACCTAAAAAT	GAAAGTGGAT	ATGATAAATT	CAAAGGACGC	2280
ACTTACTGGA	ATTTAACCTC	GAAAGTGGAT	ATGATAAATT	CAAAGGACGC	CCTCACTATT	2340
GACTCCAGAG	GAAGCGATAG	TGCAGGCACA	CTTACCCAGC	CTTATAATTT	AAACGGTATA	2400
TCATTCAACA	AAGACACTAC	CTTTAATGTT	GAACGAAATG	CAAGAGTCAA	CTTTGACATC	2460
AAGGCACCAA	TAGGGATAAA	TAAGTATTCT	AGTTTGAATT	ACGCATCATT	TAATGGAAAC	2520
ATTTTCAGTTT	CGGGAGGGGG	GAGTGTGAT	TTCACACTTC	TCGCCTCATC	CTCTAACGTC	2580
CAAACCCCCG	GTGTAGTTAT	AAATTCTAAA	TACTTTAATG	TTTCAACAGG	GTCAAGTTTA	2640
AGATTTAAAA	CTTCAGGCTC	AACAAAAACT	GGCTTCTCAA	TAGAGAAAGA	TTTAACTTTA	2700
AATGCCACCG	GAGGCAACAT	AACACTTTTG	CAAGTTGAAG	GCACCGATGG	AATGATTGGT	2760
AAAGGCATTG	TAGCCAAAAA	AAACATAACC	TTTGAAGGAG	GTAAGATGAG	GTTTGGCTCC	2820
AGGAAAGCCG	TAACAGAAAT	CGAAGGCAAT	GTTACTATCA	ATAACAACGC	TAACGTCACT	2880
CTTATCGGTT	CGGATTTTGA	CAACCATCAA	AAACCTTTAA	CTATTAAAAA	AGATGTCATC	2940
ATTAATAGCG	GCAACCTTAC	CGCTGGAGGC	AATATTGTCA	ATATAGCCGG	AAATCTTACC	3000
GTTGAAAGTA	ACGCTAATTT	CAAAGCTATC	ACAAATTTCA	CTTTTAATGT	AGGCGGCTTG	3060
TTTGACAACA	AAGGCAATTC	AAATATTTCC	ATTGCCAAAG	GAGGGGCTCG	CTTTAAAGAC	3120
ATTGATAATT	CCAAGAATTT	AAGCATCACC	ACCAACTCCA	GCTCCACTTA	CCGCACTATT	3180
ATAAGCGGCA	ATATAACCAA	TAAAAACGGT	GATTTAAATA	TTACGAACGA	AGGTAGTGAT	3240
ACTGAAATGC	AAATTGGCGG	CGATGTCTCG	CAAAAAGAAG	GTAATCTCAC	GATTTCTTCT	3300
GACAAAATCA	ATATTACCAA	ACAGATAACA	ATCAAGGCAG	GTGTTGATGG	GGAGAATTCC	3360
GATTCAGACG	CGACAAACAA	TGCCAATCTA	ACCATTAAAA	CCAAAGAATT	GAAATTAACG	3420
CAAGACCTAA	ATATTTTCAGG	TTTCAATAAA	GCAGAGATTA	CAGCTAAAGA	TGGTAGTGAT	3480
TTAACTATTG	GTAACACCAA	TAGTGCTGAT	GGTACTAATG	CCAAAAAAGT	AACCTTTAAC	3540

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GAAACATCCG	GTAGTAATAA	CAACACTGAA	GATAGCAGTG	ACAATAATGC	CGGCTTAACT	3660
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AACGTGGAGA	TAACCGCTCA	AACAGGTAGT	ATCCTAGGTG	GAATTGAGTC	CAGCTCTGGC	3840
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GTTACTGTTA	CTGCAAATAG	CGGTGCATTA	ACCACTTTGG	CAGGCTCTAC	AATTAAAGGA	3960
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ACAGTAGAGG	TTAAAGCAAC	CGAAAGTTTA	ACCACTCAAT	CCAATTCAAA	AATTAAAGCA	4080
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GGCGTAAGTG	CTGTACGTTT	TATTGAGCCA	AATAATACAA	TTACAGTCGA	TACACAAAAT	4800
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AACAGTGATG	GCGCGACGGT	GTGCGTTAAT	ATCGCTGATA	ACGGGCGGTA	GCGGTCAGTA	4920
ATTGACAAGG	TAGATTTTCA	CCTGCAATGA	AGTCATTTTA	TTTTCGTATT	ATTTACTGTG	4980
TGGGTAAAG	TTCAGTACGG	GCTTTACCCA	TCTTGTAATA	AATTACGGAG	AATACAATAA	5040
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GCCAAGTTTT	TTATAAGGCG	AGCCAGGGTT	ATAGTGAAGA	AAATATCGCT	CGTAGCCTGC	5460
CATCTTTGAA	ACAAGGAAAA	GTGTATGAAG	ATGGTCGTCA	GTGGTTTCGAT	TGCGTGAAT	5520
TCAATATGGC	AAAAGAAAAAT	CCACTTAAAG	TCACTCGCGT	GCATTACGAG	TTAAACCCTA	5580

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TTGTAAATGC	CAATTTGACC	GGACATGATG	ATGTATTAAA	TCTAAACGCA	TTGACCAATG	5760
TAAAAGCACC	ATCAAAATCT	TATGCGGTAG	GCATAGGATA	TACTTATCCG	TTTTATGATA	5820
AACACCAATC	CTTAAGTCTT	TATACCAGCA	TGAGTTATGC	TGATTCTAAT	GATATCGACG	5880
GCTTACCAAG	TGCGATTAAAT	CGTAAATTAT	CAAAAGGTCA	ATCTATCTCT	GCGAATCTGA	5940
AATGGAGTTA	TTATCTCCCG	ACATTTAACC	TTGGAATGGA	AGACCAGTTT	AAAATTAATT	6000
TAGGCTACAA	CTACCGCCAT	ATTAATCAAA	CATCCGAGTT	AAACACCCTG	GGTGCAACGA	6060
AGAAAAAATT	TGCAGTATCA	GGCGTAAGTG	CAGGCATTGA	TGGACATATC	CAATTTACCC	6120
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CTGCGGGTTT	AGGCATTAAA	ACCTCTCCTA	CACAAAACCT	AAGCTTAGAT	GCTTTTGTG	6600
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GGTAAGCGTT	CCGCCTACCA	GTTTATAACT	ATATGCTTTA	CCCGCCAATT	TACAGTCTAT	6780
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AGCAAACCAA	GCAAACCAAG	CAAACCAAGC	AAACCAAGCA	ATGCTAAAAA	ACAATTTATA	6960
TGATAAACTA	AAACATACTC	CATACCATGG	CAATACAAGG	GATTTAATAA	TATGACAAAA	7020
GAAAATTTAC	AAAGTGTTCC	ACAAAATACG	ACCGCTTCAC	TTGTAGAATC	AAACAACGAC	7080
CAAACCTCCC	TGCAAATACT	TAAACAACCA	CCCAAACCCA	ACCTATTACG	CCTGGAACAA	7140
CATGTCGCCA	AAAAAGATTA	TGAGCTTGCT	TGCCGCGAAT	TAATGGCGAT	TTTGGAaaaa	7200
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TATCTACCCG	AAAAACTACT	AATTCATTTT	GCCACTCGTC	TCGCTAATGC	AATTACAACA	7320
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CAACGCTGGT	TGACGCTGAT	TTTTGCCTCT	TCCCCCTACG	TTAACGCAGA	CCATATTCTC	7440
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AAAAAACTCG	CCGAAATTGC	TAATTTAGAT	GAATTGCCTG	CAAATATCCT	TCATGATGTA	7740
TATATGCACT	GCAGTTATGA	TTTAGCAAAA	AACAAGCACG	ATGTTAAGCG	TCCATTAAAC	7800
GAAC TTGTCC	GCAAGCATAT	CCTCACGCAA	GGATGGCAAG	ACCGCTACCT	TTACACCTTA	7860
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TCGATTTATC	GCACGCATTG	AACTTCAATG	ATTGCTGCTC	GAGAAAAATT	CTATTTAGTC	7980
GGCTTAGGCC	ATGAGGGCGT	TGATAACATA	GGTCGAGAAG	TGTTTGACGA	GTTCTTTGAA	8040
ATCAGTAGCA	ATAATATAAT	GGAGAGACTG	TTTTTTATCC	GTAAACAGTG	CGAAACTTTC	8100
CAACCCGCAG	TGTTCTATAT	GCCAAGCATT	GGCATGGATA	TTACCACGAT	TTTGTTGAGC	8160
AACACTCGGC	TTGCCCCCTAT	TCAAGCTGTA	GCCTTGGGTC	ATCCGCCCAC	TACGCATTCT	8220
GAATTTATTG	ATTATGTCAT	CGTAGAAGAT	GATTATGTGG	GCAGTGAAGA	TTGTTTTAGC	8280
GAAACCCTTT	TACGCTTACC	CAAAGATGCC	CTACCTTATG	TACCATCTGC	ACTCGCCCCA	8340
CAAAAAGTGG	ATTATGTACT	CAGGGAAAAC	CCTGAAGTAG	TCAATATCGG	TATTGCCGCT	8400
ACCACAATGA	AATTAAACCC	TGAATTTTTG	CTAACATTGC	AAGAAATCAG	AGATAAAGCT	8460
AAAGTCAAAA	TACATTTTCA	TTTCGCACTT	GGACAATCAA	CAGGCTTGAC	ACACCCTTAT	8520
GTCAAATGGT	TTATCGAAAG	CTATTTAGGT	GACGATGCCA	CTGCACATCC	CCACGCACCT	8580
TATCACGATT	ATCTGGCAAT	ATTGCGTGAT	TGCGATATGC	TACTAAATCC	GTTTCCTTTC	8640
GGTAATACTA	ACGGCATAAT	TGATATGGTT	ACATTAGGTT	TAGTTGGTGT	ATGCAAAAACG	8700
GGGGATGAAG	TACATGAACA	TATTGATGAA	GGTCTGTTTA	AACGCTTAGG	ACTACCAGAA	8760
TGGCTGATAG	CCGACACACG	AGAAACATAT	ATTGAATGTG	CTTTGCGTCT	AGCAGAAAAAC	8820
CATCAAGAAC	GCCTTGAACT	CCGTCGTTAC	ATCATAGAAA	ACAACGGCTT	ACAAAAGCTT	8880
TTTACAGGCG	ACCCTCGTCC	ATTGGGCAAA	ATACTGCTTA	AGAAAACAAA	TGAATGGAAG	8940
CGGAAGCACT	TGAGTAAAAA	ATAACGGTTT	TTTAAAGTAA	AAGTGCGGTT	AATTTTCAAA	9000
GCGTTTTTAA	AACCTCTCAA	AAATCAACCG	CACTTTTATC	TTTATAACGC	TCCCCGCGCGC	9060
TGACAGTTTA	TCTCTTTCTT	AAAATACCCA	TAAAATTGTG	GCAATAGTTG	GGTAATCAAA	9120
TTCAATTGTT	GATACGGCAA	ACTAAAGACG	GCGCGTTCTT	CGGCAGTCAT	C	9171

(2) INFORMATION FOR SEQ ID NO:6:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9323 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGCCACTTCA	ATTTTGGATT	GTTGAAATTC	AACTAACCAA	AAAGTGCGGT	TAAAATCTGT	60
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TTGGGCATTG GTTGCGGTTT CTTTTTCGGT TAATAGTAAA TTATATTCTG GACGACTATG	180
CAATCCACCA ACAACTTTAC CGTTGGTTTT AAGCGTTAAT GTAAGTTCTT GCTCTTCTTG	240
GCGAATACGT AATCCCATTT TTTGTTTAGC AAGAAAATGA TCGGGATAAT CATAATAGGT	300
GTTGCCCAAA AATAAATTTT GATGTTCTAA AATCATAAAT TTTGCAAGAT ATTGTGGCAA	360
TTCAATACCT ATTTGTGGCG AAATCGCCAA TTTTAATTCA ATTTCTTGTA GCATAATATT	420
TCCCACTCAA ATCAACTGGT TAAATATACA AGATAATAAA AATAAATCAA GATTTTTGTG	480
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AGTATAAATC CGCCATATAA AATGGTATAA TCTTTCATCT TTCATCTTTC ATCTTTCATC	600
TTTCATCTTT CATCTTTCAT CTTTCATCTT TCATCTTTC TCTTTCATCT TTCATCTTTC	660
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CTGCCACTAT TCGAAACCAA GGTAACTTT CTGCTGATTC TGTAAGCAAA GATAAAGCG	1680
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CGTTAATTGA CGGCAATATT AACGCTCAAG GTAGTGGTGA TATCGCTAAA ACCGGTGGTT	2040
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TATTTACTGT GTGGGTTAAA GTTCAGTACG GGCTTTACCC ATCTTGTAAG AAATTACGGA	5340
GAATACAATA AAGTATTTTT AACAGGTTAT TATTATGAAA AATATAAAAA GCAGATTAAA	5400
ACTCAGTGCA ATATCAGTAT TGCTTGGCCT GGCTTCTTCA TCATTGTATG CAGAAGAAGC	5460
GTTTTTAGTA AAAGGCTTTC AGTTATCTGG TGCACTTGAA ACTTTAAGTG AAGACGCCCA	5520
ACTGTCTGTA GCAAAATCTT TATCTAAATA CCAAGGCTCG CAAACTTTAA CAAACCTAAA	5580
AACAGCACAG CTTGAATTAC AGGCTGTGCT AGATAAGATT GAGCCAAATA AATTTGATGT	5640
GATATTGCCG CAACAAACCA TTACGGATGG CAATATCATG TTTGAGCTAG TCTCGAAATC	5700
AGCCCGCAGAA AGCCAAGTTT TTTATAAGGC GAGCCAGGGT TATAGTGAAG AAAATATCGC	5760
TCGTAGCCTG CCATCTTTGA AACAAGGAAA AGTGTATGAA GATGGTCGTC AGTGGTTCGA	5820
TTTGCGTGAA TTTAATATGG CAAAAGAAAA CCCGCTTAAG GTTACCCGTG TACATTACGA	5880
ACTAAACCTT AAAACAAAA CCTCTAATTT GATAATTGCG GGCTTCTCGC CTTTTGGTAA	5940
AACGCGTAGC TTTATTTCTT ATGATAATTT CGGCGCGAGA GAGTTTAACT ACCAACGTGT	6000
AAGCTTGGGT TTTGTTAATG CCAATTTAAC TGGTCATGAT GATGTGTTAA TTATACCAGT	6060
ATGAGTTATG CTGATTCTAA TGATATCGAC GGCTTACCAA GTGCGATTAA TCGTAAATTA	6120
TCAAAAGGTC AATCTATCTC TCGGAATCTG AAATGGAGTT ATTATCTCCC AACATTTAAC	6180

CTTGGCATGG	AAGACCAATT	TAAAATTAAT	TTAGGCTACA	ACTACCGCCA	TATTAATCAA	6240
ACCTCCGCGT	TAAATCGCTT	GGGTGAAACG	AAGAAAAAAT	TTGCAGTATC	AGGCGTAAGT	6300
GCAGGCATTG	ATGGACATAT	CCAATTTACC	CCTAAAACAA	TCTTTAATAT	TGATTTAACT	6360
CATCATTATT	ACGCGAGTAA	ATTACCAGGC	TCTTTTGGAA	TGGAGCGCAT	TGGCGAAACA	6420
TTTAATCGCA	GCTATCACAT	TAGCACAGCC	AGTTTAGGGT	TGAGTCAAGA	GTTTGCTCAA	6480
GGTTGGCATT	TTAGCAGTCA	ATTATCAGGT	CAATTTACTC	TACAAGATAT	TAGCAGTATA	6540
GATTTATTCT	CTGTAACAGG	TACTTATGGC	GTCAGAGGCT	TTAAATACGG	CGGTGCAAGT	6600
GGTGAGCGCG	GTCTTGTATG	GCGTAATGAA	TTAAGTATGC	CAAAATACAC	CCGCTTCCAA	6660
ATCAGCCCTT	ATGCGTTTTA	TGATGCAGGT	CAGTTCCGTT	ATAATAGCGA	AAATGCTAAA	6720
ACTTACGGCG	AAGATATGCA	CACGGTATCC	TCTGCGGGTT	TAGGCATTAA	AACCTCTCCT	6780
ACACAAAAC	TAAGCCTAGA	TGCTTTTGTT	GCTCGTCGCT	TTGCAAATGC	CAATAGTGAC	6840
AATTTGAATG	GCAACAAAAA	ACGCACAAGC	TCACCTACAA	CCTTCTGGGG	GAGATTAACA	6900
TTCAAGTTTCT	AACCCTGAAA	TTTAATCAAC	TGGTAAGCGT	TCCGCCTACC	AGTTTATAAC	6960
TATATGCTTT	ACCCGCCAAT	TTACAGTCTA	TAGGCAACCC	TGTTTTTACC	CTTATATATC	7020
AAATAAACAA	GCTAAGCTGA	GCTAAGCAAA	CCAAGCAAAC	TCAAGCAAGC	CAAGTAATAC	7080
TAAAAAAACA	ATTTATATGA	TAAACTAAAG	TATACTCCAT	GCCATGGCGA	TACAAGGGAT	7140
TTAATAATAT	GACAAAAGAA	AATTTGCAAA	ACGCTCCTCA	AGATGCGACC	GCTTTACTTG	7200
CGGAATTAAG	CAACAATCAA	ACTCCCCTGC	GAATATTTAA	ACAACCACGC	AAGCCCAGCC	7260
TATTACGCTT	GGAACAACAT	ATCGCAAAAA	AAGATTATGA	GTTTGCTTGT	CGTGAATTAA	7320
TGGTGATTCT	GGAAAAATG	GACGCTAATT	TTGGAGGCGT	TCACGATATT	GAATTTGACG	7380
CACCCGCTCA	GCTGGCATAT	CTACCCGAAA	AATTACTAAT	TTATTTTGCC	ACTCGTCTCG	7440
CTAATGCAAT	TACAACACTC	TTTTCCGACC	CCGAATTGGC	AATTTCTGAA	GAAGGGGCGT	7500
TAAAGATGAT	TAGCCTGCAA	CGCTGGTTGA	CGCTGATTTT	TGCCTCTTCC	CCCTACGTTA	7560
ACGCAGACCA	TATTCTCAAT	AAATATAATA	TCAACCCAGA	TTCCGAAGGT	GGCTTTCATT	7620
TAGCAACAGA	CAACTCTTCT	ATTGCTAAAT	TCTGTATTTT	TTACTTACCC	GAATCCAATG	7680
TCAATATGAG	TTTAGATGCG	TTATGGGCAG	GGAATCAACA	ACTTTGTGCT	TCATTGTGTT	7740
TTGCGTTGCA	GTCTTCACGT	TTTATTGGTA	CCGCATCTGC	GTTTCATAAA	AGAGCGGTGG	7800
TTTTACAGTG	GTTTCCTAAA	AAACTCGCCG	AAATTGCTAA	TTTAGATGAA	TTGCCTGCAA	7860
ATATCCTTCA	TGATGTATAT	ATGCACTGCA	GTTATGATTT	AGCAAAAAAC	AAGCACGATG	7920
TTAAGCGTCC	ATTAAACGAA	CTTGTCGGCA	AGCATATCCT	CACGCAAGGA	TGGCAAGACC	7980
GCTACCTTTA	CACCTTAGGT	AAAAAGGACG	GCAAACCTGT	GATGATGGTA	CTGCTTGAAC	8040
ATTTTAATTC	GGGACATTCT	ATTTATCGTA	CACATTCAAC	TTCAATGATT	GCTGCTCGAG	8100
AAAAATTCTA	TTTAGTCGGC	TTAGGCCATG	AGGGCGTTGA	TAAAATAGGT	CGAGAAGTGT	8160
TTGACGAGTT	CTTTGAAATC	AGTAGCAATA	ATATAATGGA	GAGACTGTTT	TTTATCCGTA	8220

AACAGTGCGA AACTTTCCAA CCCGCAGTGT TCTATATGCC AAGCATTGGC ATGGATATTA	8280
CCACGATTTT TGTGAGCAAC ACTCGGCTTG CCCCTATTCA AGCTGTAGCC CTGGGTCATC	8340
CTGCCACTAC GCATTCTGAA TTTATTGATT ATGTCATCGT AGAAGATGAT TATGTGGGCA	8400
GTGAAGATTG TTTGAGCGAA ACCCTTTTAC GCTTACCCAA AGATGCCCTA CCTTATGTAC	8460
CTTCTGCACT CGCCCCACAA AAAGTGGATT ATGTACTCAG GGAAAACCCT GAAGTAGTCA	8520
ATATCGGTAT TGCCGCTACC ACAATGAAAT TAAACCCTGA ATTTTGTGCTA ACATTGCAAG	8580
AAATCAGAGA TAAAGCTAAA GTCAAAATAC ATTTTCATTT CGCACTTGGA CAATCAACAG	8640
GCTTGACACA CCCTTATGTC AAATGGTTTA TCGAAAGCTA TTTAGGTGAC GATGCCACTG	8700
CACATCCCCA CGCACCTTAT CACGATTATC TGGCAATATT GCGTGATTGC GATATGCTAC	8760
TAAATCCGTT TCCTTTCGGT AATACTAACG GCATAATTGA TATGGTTACA TTAGGTTTAG	8820
TTGGTGTATG CAAAACGGGG GATGAAGTAC ATGAACATAT TGATGAAGGT CTGTTTAAAC	8880
GCTTAGGACT ACCAGAATGG CTGATAGCCG ACACACGAGA AACATATATT GAATGTGCTT	8940
TGCGTCTAGC AGAAAACCAT CAAGAACGCC TTGAACTCCG TCGTTACATC ATAGAAAACA	9000
ACGGCTTACA AAAGCTTTTT ACAGGCGACC CTCGTCCATT GGGCAAAATA CTGCTTAAGA	9060
AAACAAATGA ATGGAAGCGG AAGCACTTGA GTAAAAAATA ACGGTTTTTT AAAGTAAAAG	9120
TGCGGTTAAT TTTCAAAGCG TTTTAAAAAC GTCTCAAAAA TCAACCGCAC TTTTATCTTT	9180
ATAACGATCC CGCACGCTGA CAGTTTATCA GCCTCCCGCC ATAAACTCC GCCTTTCATG	9240
GCGGAGATTT TAGCCAAAAC TGGCAGAAAT TAAAGGCTAA AATCACCAAA TTGCACCACA	9300
AAATCACCAA TACCACAAA AAA	9323

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 4794 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGAACAAGA TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT TGCTGTGTCT	60
GAATTGACAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA GTGAAAAACC TGTTTCGTACG	120
AAAGTACGCC ACTTGCGGTT AAAGCCACTT TCCGCTATAT TGCTATCTTT GGGCATGGCA	180
TCCATTCCGC AATCTGTTTT AGCGAGCGGT TTACAGGGAA TGAGCGTCGT ACACGGTACA	240
GCAACCATGC AAGTAGACGG CAATAAAACC ACTATCCGTA ATAGCGTCAA TGCTATCATC	300
AATTGGAAAC AATTTAACAT TGACCAAAAT GAAATGGTGC AGTTTTTACA AGAAAGCAGC	360
AACTCTGCCG TTTTCAACCG TGTTACATCT GACCAAATCT CCAATTAAA AGGGATTTTA	420

GATTCTAACG	GACAAGTCTT	TTTAATCAAC	CCAAATGGTA	TCACAATAGG	TAAAGACGCA	480
ATTATTAACA	CTAATGGCTT	TACTGCTTCT	ACGCTAGACA	TTTCTAACGA	AAACATCAAG	540
GCGCGTAATT	TCACCCTTGA	GCAAACCAAG	GATAAAGCAC	TCGCTGAAAT	CGTGAATCAC	600
GGTTTAATTA	CCGTTGGTAA	AGACGGTAGC	GTAAACCTTA	TTGGTGGCAA	AGTGAAAAAC	660
GAGGGCGTGA	TTAGCGTAAA	TGGCGGTAGT	ATTTCTTTAC	TTGCAGGGCA	AAAAATCACC	720
ATCAGCGATA	TAATAAATCC	AACCATCACT	TACAGCATTG	CTGCACCTGA	AAACGAAGCG	780
ATCAATCTGG	GCGATATTTT	TGCCAAAGGT	GGTAACATTA	ATGTCCGCGC	TGCCACTATT	840
CGCAATAAAG	GTAAACTTTC	TGCCGACTCT	GTAAGCAAAG	ATAAAAGTGG	TAACATTGTT	900
CTCTCTGCCA	AAGAAGGTGA	AGCGGAAATT	GGCGGTGTAA	TTCCGCTCA	AAATCAGCAA	960
GCCAAAGGTG	GTAAGTTGAT	GATTACAGGC	GATAAAGTTA	CATTGAAAAC	GGGTGCAGTT	1020
ATCGACCTTT	CGGGTAAAGA	AGGGGGAGAA	ACTTATCTTG	GCGGTGACGA	GCGTGGCGAA	1080
GGTAAAAACG	GCATTCAATT	AGCAAAGAAA	ACCACTTTAG	AAAAAGGCTC	AACAATTAAT	1140
GTGTCAGGTA	AAGAAAAAGG	TGGGCGCGCT	ATTGTATGGG	GCGATATTGC	GTTAATTGAC	1200
GGCAATATTA	ATGCCCAAGG	TAAAGATATC	GCTAAACTG	GTGGTTTTGT	GGAGACGTCG	1260
GGGCATTACT	TATCCATTGA	TGATAACGCA	ATTGTTAAAA	CAAAGAATG	GCTACTAGAC	1320
CCAGAGAATG	TGACTATTGA	AGCTCCTTCC	GCTTCTCGCG	TCGAGCTGGG	TGCCGATAGG	1380
AATTCCCACT	CGGCAGAGGT	GATAAAAGTG	ACCCTAAAAA	AAAATAACAC	CTCCTTGACA	1440
ACACTAACCA	ATACAACCAT	TTCAAATCTT	CTGAAAAGTG	CCCACGTGGT	GAACATAACG	1500
GCAAGGAGAA	AACTTACCGT	TAATAGCTCT	ATCAGTATAG	AAAGAGGCTC	CCACTTAATT	1560
CTCCACAGTG	AAGGTCAGGG	CGGTCAAGGT	GTTCAGATTG	ATAAAGATAT	TACTTCTGAA	1620
GGCGGAAATT	TAACCATTTA	TTCTGGCGGA	TGGGTTGATG	TCATAAAAA	TATTACGCTT	1680
GGTAGCGGCT	TTTTAAACAT	CACAACTAAA	GAAGGAGATA	TCGCCTTCGA	AGACAAGTCT	1740
GGACGGAAAC	ACCTAACCAT	TACAGCCCAA	GGGACCATCA	CCTCAGGTAA	TAGTAACGGC	1800
TTTAGATTTA	ACAACGTCTC	TCTAAACAGC	CTTGGCGGAA	AGCTGAGCTT	TACTGACAGC	1860
AGAGAGGACA	GAGGTAGAAG	AACTAAGGGT	AATATCTCAA	ACAAATTTGA	CGGAACGTTA	1920
AACATTTCCG	GAAGTGTAGA	TATCTCAATG	AAAGCACCCA	AAGTCAGCTG	GTTTTACAGA	1980
GACAAAGGAC	GCACCTACTG	GAACGTAACC	ACTTTAAATG	TTACCTCGGG	TAGTAAATTT	2040
AACCTCTCCA	TTGACAGCAC	AGGAAGTGGC	TCAACAGGTC	CAAGCATACG	CAATGCAGAA	2100
TTAAATGGCA	TAACATTTAA	TAAAGCCACT	TTTAATATCG	CACAAGGCTC	AACAGCTAAC	2160
TTTAGCATCA	AGGCATCAAT	AATGCCCTTT	AAGAGTAACG	CTAACTACGC	ATTATTTAAT	2220
GAAGATATTT	CAGTCTCAGG	GGGGGGTAGC	CTTAATTTCA	AACTTAACGC	CTCATCTAGC	2280
AACATACAAA	CCCCTGGCGT	AATTATAAAA	TCTCAAAACT	TTAATGTCTC	AGGAGGGTCA	2340
ACTTTAAATC	TCAAGGCTGA	AGGTTCAACA	GAAACCGCTT	TTTCAATAGA	AAATGATTTA	2400
AACTTAAACG	CCACCGGTGG	CAATATAACA	ATCAGACAAG	TCGAGGGTAC	CGATTACGCG	2460

GTCAACAAAG GTGTCGCAGC CAAAAAAAC ATAACCTTTTA AAGGGGGTAA TATCACCTTC	2520
GGCTCTCAAA AAGCCACAAC AGAAATCAAA GGCAATGTTA CCATCAATAA AAACACTAAC	2580
GCTACTCTTT GTGGTGCGAA TTTTGCCGAA AACAAATCGC CTTTAAATAT AGCAGGAAAT	2640
GTTATTAATA ATGGCAACCT TACCACTGCC GGCTCCATTA TCAATATAGC CGGAAATCTT	2700
ACTGTTTCAA AAGGCGCTAA CCTTCAAGCT ATAACAAATT ACACCTTTAA TGTAGCCGGC	2760
TCATTTGACA ACAATGGCGC TTCAAACATT TCCATTGCCA GAGGAGGGGC TAAATTTAAA	2820
GATATCAATA ACACCAGTAG CTTAAATATT ACCACCAACT CTGATACCAC TTACCGCACC	2880
ATTATAAAAG GCAATATATC CAACAAATCA GGTGATTGA ATATTATTGA TAAAAAAGC	2940
GACGCTGAAA TCCAAATTGG CGGCAATATC TCACAAAAAG AAGGCAATCT CACAATTTCT	3000
TCTGATAAAG TAAATATTAC CAATCAGATA ACAATCAAAG CAGGCGTTGA AGGGGGGCGT	3060
TCTGATTCAA GTGAGGCAGA AAATGCTAAC CTAACCTATTC AAACCAAAGA GTTAAAATTG	3120
GCAGGAGACC TAAATATTTT AGGCTTTAAT AAAGCAGAAA TTACAGCTAA AAATGGCAGT	3180
GATTTAACTA TTGGCAATGC TAGCGGTGGT AATGCTGATG CTAAAAAAGT GACTTTTGAC	3240
AAGGTTAAAG ATTCAAAAAT CTCGACTGAC GGTCACAATG TAACACTAAA TAGCGAAGTG	3300
AAAACGTCTA ATGGTAGTAG CAATGCTGGT AATGATAACA GCACCGGTTT AACCATTTC	3360
GCAAAAGATG TAACGGTAAA CAATAACGTT ACCTCCCACA AGACAATAAA TATCTCTGCC	3420
GCAGCAGGAA ATGTAACAAC CAAAGAAGGC ACAACTATCA ATGCAACCAC AGGCAGCGTG	3480
GAAGTAACTG CTCAAAATGG TACAATTAAA GGCAACATTA CCTCGCAAAA TGTAACAGTG	3540
ACAGCAACAG AAAATCTTGT TACCACAGAG AATGCTGTCA TTAATGCAAC CAGCGGCACA	3600
GTAAACATTA GTACAAAAAC AGGGGATATT AAAGGTGGAA TTGAATCAAC TTCCGGTAAT	3660
GTAAATATTA CAGCGAGCGG CAATACACTT AAGSTAAGTA ATATCACTGG TCAAGATGTA	3720
ACAGTAACAG CGGATGCAGG AGCCTTGACA ACTACAGCAG GCTCAACCAT TAGTGCGACA	3780
ACAGGCAATG CAAATATTAC AACCAAAACA GGTGATATCA ACGGTAAAGT TGAATCCAGC	3840
TCCGGCTCTG TAACACTTGT TGCAACTGGA GCAACTCTTG CTGTAGGTAA TATTTAGGT	3900
AACACTGTTA CTATTACTGC SGATAGCGGT AAATTAACCT CCACAGTAGG TTCTACAATT	3960
AATGGGACTA ATAGTGTAAC CACCTCAAGC CAATCAGGCG ATATTGAAGG TACAAATTTCT	4020
GGTAATACAG TAAATGTTAC AGCAAGCACT GGTGATTAA CTATTGGAAA TAGTGCAAAA	4080
GTTGAAGCGA AAAATGGAGC TGCAACCTTA ACTGCTGAAT CAGGCAAATT AACCACCCAA	4140
ACAGGCTCTA GCATTACCTC AAGCAATGGT CAGACAATC TTACAGCCAA GGATAGCAGT	4200
ATCGCAGGAA ACATTAATGC TGCTAATGTG ACGTTAAATA CCACAGGCAC TTTAACTACT	4260
ACAGGGGATT CAAAGATTAA CGCAACCAGT GGTACCTTAA CAATCAATGC AAAAGATGCC	4320
AAATTAGATG GTGCTGCATC AGGTGACCGC ACAGTAGTAA ATGCAACTAA CGCAAGTGGC	4380
TCTGGTAACG TGA CTGCGAA AACCTCAAGC AGCGTGAATA TCACCGGGGA TTTAAACACA	4440
ATAAATGGGT TAAATATCAT TTCGGAAAAT GGTAGAAACA CTGTGCGCTT AAGAGGCAAG	4500

GAAATTGATG	TGAAATATAT	CCAACCAGGT	GTAGCAAGCG	TAGAAGAGGT	AATTGAAGCG	4560
AAACGCGTCC	TTGAGAAGGT	AAAAGATTTA	TCTGATGAAG	AAAGAGAAAC	ACTAGCCAAA	4620
CTTGGTGTAA	GTGCTGTACG	TTTCGTTGAG	CCAAATAATG	CCATTACGGT	TAATACACAA	4680
AACGAGTTTA	CAACCAAACC	ATCAAGTCAA	GTGACAATTT	CTGAAGGTAA	GGCGTGTTTC	4740
TCAAGTGGTA	ATGGCGCACG	AGTATGTACC	AATGTTGCTG	ACGATGGACA	GCAG	4794

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 4803 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGAACAAGA	TATATCGTCT	CAAATTCAGC	AAACGCCTGA	ATGCTTTGGT	TGCTGTGTCT	60
GAATTGACAC	GGGGTTGTGA	CCATTCCACA	GAAAAAGGCA	GTGAAAAACC	TGTTTCGTACG	120
AAAGTACGCC	ACTTGGCGTT	AAAGCCACTT	TCCGCTATAT	TGCTATCTTT	GGGCATGGCA	180
TCCATTCCGC	AATCTGTTTT	AGCGAGCGGT	TTACAGGGAA	TGAGCGTCGT	ACACGGTACA	240
GCAACCATGC	AAGTAGACGG	CAATAAAACC	ACTATCCGTA	ATAGCGTCAA	TGCTATCATC	300
AATTGGAAAC	AATTTAACAT	TGACCAAAT	GAAATGGTGC	AGTTTTTACA	AGAAAGCAGC	360
AACTCTGCCG	TTTTCAACCG	TGTTACATCT	GACCAAATCT	CCCAATTAAA	AGGGATTTTA	420
GATTCTAACG	GACAAGTCTT	TTTAATCAAC	CCAAATGGTA	TCACAATAGG	TAAAGACGCA	480
ATTATTAACA	CTAATGGCTT	TACTGCTTCT	ACGCTAGACA	TTTCTAACGA	AAACATCAAG	540
GCGCGTAATT	TCACCCTTGA	GCAAACCAAG	GATAAAGCAC	TCGCTGAAAT	CGTGAATCAC	600
GGTTTAATTA	CCGTTGGTAA	AGACGGTAGC	GTAAACCTTA	TTGGTGGCAA	AGTGAAAAAC	660
GAGGGCGTGA	TTAGCGTAAA	TGGCGGTAGT	ATTTCTTTAC	TTGCAGGGCA	AAAAATCACC	720
ATCAGCGATA	TAATAAATCC	AACCATCACT	TACAGCATTG	CTGCACCTGA	AAACGAAGCG	780
ATCAATCTGG	GCGATATTTT	TGCCAAAGGT	GGTAACATTA	ATGTCCGCGC	TGCCACTATT	840
CGCAATAAAG	GTAAACTTTC	TGCCGACTCT	GTAAGCAAAG	ATAAAAGTGG	TAACATTGTT	900
CTCTCTGCCA	AAGAAGGTGA	AGCGGAAATT	GGCGGTGTAA	TTCCGCTCA	AAATCAGCAA	960
GCCAAAGGTG	GTAAGTTGAT	GATTACAGGT	GATAAAGTCA	CATTAAAAAC	AGGTGCAGTT	1020
ATCGACCTTT	CAGGTAAAGA	AGGGGGAGAG	ACTTATCTTG	GCGGTGATGA	GCGTGGCGAA	1080
GGTAAAAATG	GTATTCAATT	AGCGAAGAAA	ACCTCTTTAG	AAAAAGGCTC	GACAATTAAT	1140
GTATCAGGCA	AAGAAAAAGG	CGGGCGCGCT	ATTGTATGGG	GCGATATTGC	ATTAATTAAT	1200
GGTAACATTA	ATGCTCAAGG	TAGCGATATT	GCTAAAACTG	GCGGCTTTGT	GGAAACATCA	1260

GGACATGACT TATCCATTGG TGATGATGTG ATTGTTGACG CTAAAGAGTG GTTATTAGAC	1320
CCAGATGATG TGTCCATTGA AACTCTTACA TCTGGACGCA ATAATACCGG CGAAAACCAA	1380
GGATATACAA CAGGAGATGG GACTAAAGAG TCACCTAAAG GTAATAGTAT TTCTAAACCT	1440
ACATTAAACAA ACTCAACTCT TGAGCAAATC CTAAGAAGAG GTTCTTATGT TAATATCACT	1500
GCTAATAATA GAATTTATGT TAATAGCTCC ATCAACTTAT CTAATGGCAG TTAAACACTT	1560
CACACTAAAC GAGATGGAGT TAAAATTAAC GGTGATATTA CCTCAAACGA AAATGGTAAT	1620
TTAACCATT AAGCAGGCTC TTGGGTTGAT GTTCATAAAA ACATCACGCT TGGTACGGGT	1680
TTTTTTGAATA TTGTCGCTGG GGATTCTGTA GCTTTTGAGA GAGAGGGCGA TAAAGCACGT	1740
AACGCAACAG ATGCTCAAAT TACCGCACAA GGGACGATAA CCGTCAATAA AGATGATAAA	1800
CAATTTAGAT TCAATAATGT ATCTATTAAC GGGACGGGCA AGGGTTTAAA GTTTATTGCA	1860
AATCAAAATA ATTTCACTCA TAAATTTGAT GGCGAATTA ACATATCTGG AATAGTAACA	1920
ATTAACCAAA CCACGAAAAA AGATGTTAAA TACTGGAATG CATCAAAAGA CTCTTACTGG	1980
AATGTTTCTT CTCTTACTTT GAATACGGTG CAAAATTTA CTTTTATAAA ATTCGTTGAT	2040
AGCGGCTCAA ATTCCCAAGA TTTGAGGTCA TCACGTAGAA GTTTTGCAGG CGTACATTTT	2100
AACGGCATCG GAGGCAAAAC AAACCTCAAC ATCGGAGCTA ACGCAAAAGC CTTATTTAAA	2160
TTAAAACCAA ACGCCGCTAC AGACCCAAAA AAAGAATTAC CTATTACTTT TAACGCCAAC	2220
ATTACAGCTA CCGGTAACAG TGATAGCTCT GTGATGTTTG ACATACACGC CAATCTTACC	2280
TCTAGAGCTG CCGGCATAAA CATGGATTCA ATTAACATTA CCGGCGGGCT TGACTTTTCC	2340
ATAACATCCC ATAATCGCAA TAGTAATGCT TTTGAAATCA AAAAAGACTT AACTATAAAT	2400
GCAACTGGCT CGAATTTTAG TCTTAAGCAA ACGAAAGATT CTTTTTATAA TGAATACAGC	2460
AAACACGCCA TTAACCTAAG TCATAATCTA ACCATTCTTG GCGGCAATGT CACTCTAGGT	2520
GGGGAAAATT CAAGCAGTAG CATTACGGGC AATATCAATA TCACCAATAA AGCAAATGTT	2580
ACATTACAAG CTGACACCAG CAACAGCAAC ACAGGCTTGA AGAAAAGAAC TCTAACTCTT	2640
GGCAATATAT CTGTTGAGGG GAATTTAAGC CTAAGTGGTG CAAATGCAAA CATGTGCGGC	2700
AATCTTTCTA TTGCAGAAGA TTCCACATTT AAAGGAGAAG CCAGTGACAA CCTAAACATC	2760
ACCGGCACCT TTACCAACAA CGGTACCGCC AACATTAATA TAAACAAGG AGTGGTAAAA	2820
CTCCAAGGCG ATATTATCAA TAAAGGTGGT TTAAATATCA CTAATAACGC CTCAGGCACT	2880
CAAAAAACCA TTATTAACGG AAATATAACT AACGAAAAAG GCGACTTAAA CATCAAGAAT	2940
ATTAAAGCCG ACGCCGAAAT CCAAATTGGC GGCAATATCT CAAAAAAGA AGGCAATCTC	3000
ACAATTTCTT CTGATAAAGT AAATATTACC AATCAGATAA CAATCAAAGC AGGCGTTGAA	3060
GGGGGGCGTT CTGATTCAAG TGAGGCAGAA AATGCTAACC TAACTATTCA AACCAAAGAG	3120
TTAAAATTGG CAGGAGACCT AAATATTTCA GGCTTTAATA AAGCAGAAAT TACAGCTAAA	3180
AATGGCAGTG ATTTAACTAT TGGCAATGCT AGCGGTGGTA ATGCTGATGC TAAAAAAGTG	3240
ACTTTTGACA AGGTAAAGA TTCAAAAATC TCGACTGACG GTCACAATGT AACACTAAAT	3300

AGCGAAGTGA	AAACGTCTAA	TGGTAGTAGC	AATGCTGGTA	ATGATAACAG	CACCGGTTTA	3360
ACCATTTCGG	CAAAAGATGT	AACGGTAAAC	AATAACGTTA	CCTCCCACAA	GACAATAAAT	3420
ATCTCTGCCG	CAGCAGGAAA	TGTAACAACC	AAAGAAGGCA	CAACTATCAA	TGCAACCACA	3480
GGCAGCGTGG	AAGTAACTGC	TCAAAATGGT	ACAATTAAAG	GCAACATTAC	CTCGCAAAAT	3540
GTAACAGTGA	CAGCAACAGA	AAATCTTGTT	ACCACAGAGA	ATGCTGTCAT	TAATGCAACC	3600
AGCGGCACAG	TAAACATTAG	TACAAAAACA	GGGGATATTA	AAGGTGGAAT	TGAATCAACT	3660
TCCGGTAATG	TAAATATTAC	AGCGAGCGGC	AATACACTTA	AGGTAAGTAA	TATCACTGGT	3720
CAAGATGTAA	CAGTAACAGC	GGATGCAGGA	GCCTTGACAA	CTACAGCAGG	CTCAACCATT	3780
AGTGCGACAA	CAGGCAATGC	AAATATTACA	ACCAAAACAG	GTGATATCAA	CGGTAAAGTT	3840
GAATCCAGCT	CCGGCTCTGT	AACACTTGTT	GCAACTGGAG	CAACTCTTGC	TGTAGGTAAT	3900
ATTTCAAGTA	ACACTGTTAC	TATTACTGCG	GATAGCGGTA	AATTAACCTC	CACAGTAGGT	3960
TCTACAATTA	ATGGGACTAA	TAGTGTAACC	ACCTCAAGCC	AATCAGGCGA	TATTGAAGGT	4020
ACAATTTCTG	GTAATACAGT	AAATGTTACA	GCAAGCACTG	GTGATTTAAC	TATTGGAAAT	4080
AGTGCAAAAG	TTGAAGCGAA	AAATGGAGCT	GCAACCTTAA	CTGCTGAATC	AGGCAAATTA	4140
ACCACCCAAA	CAGGCTCTAG	CATTACCTCA	AGCAATGGTC	AGACAACTCT	TACAGCCAAG	4200
GATAGCAGTA	TCGCAGGAAA	CATTAATGCT	GCTAATGTGA	CGTTAAATAC	CACAGGCACT	4260
TTAACTACTA	CAGGGGATTG	AAAGATTAA	GCAACCAGTG	GTACCTTAAC	AATCAATGCA	4320
AAAGATGCCA	AATTAGATGG	TGCTGCATCA	GGTGACCGCA	CAGTAGTAAA	TGCAACTAAC	4380
GCAAGTGGCT	CTGGTAACGT	GA CTGCGAAA	ACCTCAAGCA	GCGTGAATAT	CACCGGGGAT	4440
TTAAACACAA	TAAATGGGTT	AAATATCATT	TCGGAAAATG	GTAGAAACAC	TGTGCGCTTA	4500
AGAGGCAAGG	AAATTGATGT	GAAATATATC	CAACCAGGTG	TAGCAAGCGT	AGAAGAGGTA	4560
ATTGAAGCGA	AACGCGTCCT	TGAGAAGGTA	AAAGATTTAT	CTGATGAAGA	AAGAGAAACA	4620
CTAGCCAAAC	TTGGTGTAAG	TGCTGTACGT	TTCGTTGAGC	CAAATAATGC	CATTACGGTT	4680
AATACACAAA	ACGAGTTTAC	AACCAAACCA	TCAAGTCAAG	TGACAATTTT	TGAAGGTAAG	4740
GCGTGTTTCT	CAAGTGGTAA	TGGCGCACGA	GTATGTACCA	ATGTTGCTGA	CGATGGACAG	4800
CAG						4803

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1599 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Asn Lys Ile Tyr Arg Leu Lys Phe Ser Lys Arg Leu Asn Ala Leu
 1 5 10 15
 Val Ala Val Ser Glu Leu Thr Arg Gly Cys Asp His Ser Thr Glu Lys
 20 25 30
 Gly Ser Glu Lys Pro Val Arg Thr Lys Val Arg His Leu Ala Leu Lys
 35 40 45
 Pro Leu Ser Ala Ile Leu Leu Ser Leu Gly Met Ala Ser Ile Pro Gln
 50 55 60
 Ser Val Leu Ala Ser Gly Leu Gln Gly Met Ser Val Val His Gly Thr
 65 70 75 80
 Ala Thr Met Gln Val Asp Gly Asn Lys Thr Thr Ile Arg Asn Ser Val
 85 90 95
 Asn Ala Ile Ile Asn Trp Lys Gln Phe Asn Ile Asp Gln Asn Glu Met
 100 105 110
 Glu Gln Phe Leu Gln Glu Ser Ser Asn Ser Ala Val Phe Asn Arg Val
 115 120 125
 Thr Ser Asp Gln Ile Ser Gln Leu Lys Gly Ile Leu Asp Ser Asn Gly
 130 135 140
 Gln Val Phe Leu Ile Asn Pro Asn Gly Ile Thr Ile Gly Lys Asp Ala
 145 150 155 160
 Ile Ile Asn Thr Asn Gly Phe Thr Ala Ser Thr Leu Asp Ile Ser Asn
 165 170 175
 Glu Asn Ile Lys Ala Arg Asn Phe Thr Leu Glu Gln Thr Lys Asp Lys
 180 185 190
 Ala Leu Ala Glu Ile Val Asn His Gly Leu Ile Thr Val Gly Lys Asp
 195 200 205
 Gly Ser Val Asn Leu Ile Gly Gly Lys Val Lys Asn Glu Gly Val Ile
 210 215 220
 Ser Val Asn Gly Gly Ser Ile Ser Leu Leu Ala Gly Gln Lys Ile Thr
 225 230 235 240
 Ile Ser Asp Ile Ile Asn Pro Thr Ile Thr Tyr Ser Ile Ala Ala Pro
 245 250 255
 Glu Asn Glu Ala Ile Asn Leu Gly Asp Ile Phe Ala Lys Gly Gly Asn
 260 265 270
 Ile Asn Val Arg Ala Ala Thr Ile Arg Asn Lys Gly Lys Leu Ser Ala
 275 280 285
 Asp Ser Val Ser Lys Asp Lys Ser Gly Asn Ile Val Leu Ser Ala Lys
 290 295 300
 Glu Gly Glu Ala Glu Ile Gly Gly Val Ile Ser Ala Gln Asn Gln Gln
 305 310 315 320
 Ala Lys Gly Gly Lys Leu Met Ile Thr Gly Asp Lys Val Thr Leu Lys
 325 330 335

Thr Gly Ala Val Ile Asp Leu Ser Gly Lys Glu Gly Gly Glu Thr Tyr
 340 345 350
 Leu Gly Gly Asp Glu Arg Gly Glu Gly Lys Asn Gly Ile Gln Leu Ala
 355 360 365
 Lys Lys Thr Thr Leu Glu Lys Gly Ser Thr Ile Asn Val Ser Gly Lys
 370 375 380
 Glu Lys Gly Gly Arg Ala Ile Val Trp Gly Asp Ile Ala Leu Ile Asp
 385 390 395 400
 Gly Asn Ile Asn Ala Gln Gly Lys Asp Ile Ala Lys Thr Gly Gly Phe
 405 410 415
 Val Glu Thr Ser Gly His Tyr Leu Ser Ile Asp Asp Asn Ala Ile Val
 420 425 430
 Lys Thr Lys Glu Trp Leu Leu Asp Pro Glu Asn Val Thr Ile Glu Ala
 435 440 445
 Pro Ser Ala Ser Arg Val Glu Leu Gly Ala Asp Arg Asn Ser His Ser
 450 455 460
 Ala Glu Val Ile Lys Val Thr Leu Lys Lys Asn Asn Thr Ser Leu Thr
 465 470 475 480
 Thr Leu Thr Asn Thr Thr Ile Ser Asn Leu Leu Lys Ser Ala His Val
 485 490 495
 Val Asn Ile Thr Ala Arg Arg Lys Leu Thr Val Asn Ser Ser Ile Ser
 500 505 510
 Ile Glu Arg Gly Ser His Leu Ile Leu His Ser Glu Gly Gln Gly Gly
 515 520 525
 Gln Gly Val Gln Ile Asp Lys Asp Ile Thr Ser Glu Gly Gly Asn Leu
 530 535 540
 Thr Ile Tyr Ser Gly Gly Trp Val Asp Val His Lys Asn Ile Thr Leu
 545 550 555 560
 Gly Ser Gly Phe Leu Asn Ile Thr Thr Lys Glu Gly Asp Ile Ala Phe
 565 570 575
 Glu Asp Lys Ser Gly Arg Asn Asn Leu Thr Ile Thr Ala Gln Gly Thr
 580 585 590
 Ile Thr Ser Gly Asn Ser Asn Gly Phe Arg Phe Asn Asn Val Ser Leu
 595 600 605
 Asn Ser Leu Gly Gly Lys Leu Ser Phe Thr Asp Ser Arg Glu Asp Arg
 610 615 620
 Gly Arg Arg Thr Lys Gly Asn Ile Ser Asn Lys Phe Asp Gly Thr Leu
 625 630 635 640
 Asn Ile Ser Gly Thr Val Asp Ile Ser Met Lys Ala Pro Lys Val Ser
 645 650 655
 Trp Phe Tyr Arg Asp Lys Gly Arg Thr Tyr Trp Asn Val Thr Thr Leu
 660 665 670
 Asn Val Thr Ser Gly Ser Lys Phe Asn Leu Ser Ile Asp Ser Thr Gly
 675 680 685

95

Ser Gly Ser Thr Gly Pro Ser Ile Arg Asn Ala Glu Leu Asn Gly Ile
 690 695 700
 Thr Phe Asn Lys Ala Thr Phe Asn Ile Ala Gln Gly Ser Thr Ala Asn
 705 710 715 720
 Phe Ser Ile Lys Ala Ser Ile Met Pro Phe Lys Ser Asn Ala Asn Tyr
 725 730 735
 Ala Leu Phe Asn Glu Asp Ile Ser Val Ser Gly Gly Gly Ser Val Asn
 740 745 750
 Phe Lys Leu Asn Ala Ser Ser Ser Asn Ile Gln Thr Pro Gly Val Ile
 755 760 765
 Ile Lys Ser Gln Asn Phe Asn Val Ser Gly Gly Ser Thr Leu Asn Leu
 770 775 780
 Lys Ala Glu Gly Ser Thr Glu Thr Ala Phe Ser Ile Glu Asn Asp Leu
 785 790 795 800
 Asn Leu Asn Ala Thr Gly Gly Asn Ile Thr Ile Arg Gln Val Glu Gly
 805 810 815
 Thr Asp Ser Arg Val Asn Lys Gly Val Ala Ala Lys Lys Asn Ile Thr
 820 825 830
 Phe Lys Gly Gly Asn Ile Thr Phe Gly Ser Gln Lys Ala Thr Thr Glu
 835 840 845
 Ile Lys Gly Asn Val Thr Ile Asn Lys Asn Thr Asn Ala Thr Leu Arg
 850 855 860
 Gly Ala Asn Phe Ala Glu Asn Lys Ser Pro Leu Asn Ile Ala Gly Asn
 865 870 875 880
 Val Ile Asn Asn Gly Asn Leu Thr Thr Ala Gly Ser Ile Ile Asn Ile
 885 890 895
 Ala Gly Asn Leu Thr Val Ser Lys Gly Ala Asn Leu Gln Ala Ile Thr
 900 905 910
 Asn Tyr Thr Phe Asn Val Ala Gly Ser Phe Asp Asn Asn Gly Ala Ser
 915 920 925
 Asn Ile Ser Ile Ala Arg Gly Gly Ala Lys Phe Lys Asp Ile Asn Asn
 930 935 940
 Thr Ser Ser Leu Asn Ile Thr Thr Asn Ser Asp Thr Thr Tyr Arg Thr
 945 950 955 960
 Ile Ile Lys Gly Asn Ile Ser Asn Lys Ser Gly Asp Leu Asn Ile Ile
 965 970 975
 Asp Lys Lys Ser Asp Ala Glu Ile Gln Ile Gly Gly Asn Ile Ser Gln
 980 985 990
 Lys Glu Gly Asn Leu Thr Ile Ser Ser Asp Lys Val Asn Ile Thr Asn
 995 1000 1005
 Gln Ile Thr Ile Lys Ala Gly Val Glu Gly Gly Arg Ser Asp Ser Ser
 1010 1015 1020
 Glu Ala Glu Asn Ala Asn Leu Thr Ile Gln Thr Lys Glu Leu Lys Leu
 1025 1030 1035 1040

Ala Gly Asp Leu Asn Ile Ser Gly Phe Asn Lys Ala Glu Ile Thr Ala
 1045 1050 1055
 Lys Asn Gly Ser Asp Leu Thr Ile Gly Asn Ala Ser Gly Gly Asn Ala
 1060 1065 1070
 Asp Ala Lys Lys Val Thr Phe Asp Lys Val Lys Asp Ser Lys Ile Ser
 1075 1080 1085
 Thr Asp Gly His Asn Val Thr Leu Asn Ser Glu Val Lys Thr Ser Asn
 1090 1095 1100
 Gly Ser Ser Asn Ala Gly Asn Asp Asn Ser Thr Gly Leu Thr Ile Ser
 1105 1110 1115 1120
 Ala Lys Asp Val Thr Val Asn Asn Asn Val Thr Ser His Lys Thr Ile
 1125 1130 1135
 Asn Ile Ser Ala Ala Ala Gly Asn Val Thr Thr Lys Glu Gly Thr Thr
 1140 1145 1150
 Ile Asn Ala Thr Thr Gly Ser Val Glu Val Thr Ala Gln Asn Gly Thr
 1155 1160 1165
 Ile Lys Gly Asn Ile Thr Ser Gln Asn Val Thr Val Thr Ala Thr Glu
 1170 1175 1180
 Asn Leu Val Thr Thr Glu Asn Ala Val Ile Asn Ala Thr Ser Gly Thr
 1185 1190 1195 1200
 Val Asn Ile Ser Thr Lys Thr Gly Asp Ile Lys Gly Gly Ile Glu Ser
 1205 1210 1215
 Thr Ser Gly Asn Val Asn Ile Thr Ala Ser Gly Asn Thr Leu Lys Val
 1220 1225 1230
 Ser Asn Ile Thr Gly Gln Asp Val Thr Val Thr Ala Asp Ala Gly Ala
 1235 1240 1245
 Leu Thr Thr Thr Ala Gly Ser Thr Ile Ser Ala Thr Thr Gly Asn Ala
 1250 1255 1260
 Asn Ile Thr Thr Lys Thr Gly Asp Ile Asn Gly Lys Val Glu Ser Ser
 1265 1270 1275 1280
 Ser Gly Ser Val Thr Leu Val Ala Thr Gly Ala Thr Leu Ala Val Gly
 1285 1290 1295
 Asn Ile Ser Gly Asn Thr Val Thr Ile Thr Ala Asp Ser Gly Lys Leu
 1300 1305 1310
 Thr Ser Thr Val Gly Ser Thr Ile Asn Gly Thr Asn Ser Val Thr Thr
 1315 1320 1325
 Ser Ser Gln Ser Gly Asp Ile Glu Gly Thr Ile Ser Gly Asn Thr Val
 1330 1335 1340
 Asn Val Thr Ala Ser Thr Gly Asp Leu Thr Ile Gly Asn Ser Ala Lys
 1345 1350 1355 1360
 Val Glu Ala Lys Asn Gly Ala Ala Thr Leu Thr Ala Glu Ser Gly Lys
 1365 1370 1375
 Leu Thr Thr Gln Thr Gly Ser Ser Ile Thr Ser Ser Asn Gly Gln Thr
 1380 1385 1390

97

Thr Leu Thr Ala Lys Asp Ser Ser Ile Ala Gly Asn Ile Asn Ala Ala
 1395 1400 1405
 Asn Val Thr Leu Asn Thr Thr Gly Thr Leu Thr Thr Thr Gly Asp Ser
 1410 1415 1420
 Lys Ile Asn Ala Thr Ser Gly Thr Leu Thr Ile Asn Ala Lys Asp Ala
 1425 1430 1435 1440
 Lys Leu Asp Gly Ala Ala Ser Gly Asp Arg Thr Val Val Asn Ala Thr
 1445 1450 1455
 Asn Ala Ser Gly Ser Gly Asn Val Thr Ala Lys Thr Ser Ser Ser Val
 1460 1465 1470
 Asn Ile Thr Gly Asp Leu Asn Thr Ile Asn Gly Leu Asn Ile Ile Ser
 1475 1480 1485
 Glu Asn Gly Arg Asn Thr Val Arg Leu Arg Gly Lys Glu Ile Asp Val
 1490 1495 1500
 Lys Tyr Ile Gln Pro Gly Val Ala Ser Val Glu Glu Val Ile Glu Ala
 1505 1510 1515 1520
 Lys Arg Val Leu Glu Lys Val Lys Asp Leu Ser Asp Glu Glu Arg Glu
 1525 1530 1535
 Thr Leu Ala Lys Leu Gly Val Ser Ala Val Arg Phe Val Glu Pro Asn
 1540 1545 1550
 Asn Ala Ile Thr Val Asn Thr Gln Asn Glu Phe Thr Thr Lys Pro Ser
 1555 1560 1565
 Ser Gln Val Thr Ile Ser Glu Gly Lys Ala Cys Phe Ser Ser Gly Asn
 1570 1575 1580
 Gly Ala Arg Val Cys Thr Asn Val Ala Asp Asp Gly Gln Gln Pro
 1585 1590 1595

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1600 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Asn Lys Ile Tyr Arg Leu Lys Phe Ser Lys Arg Leu Asn Ala Leu
 1 5 10 15
 Val Ala Val Ser Glu Leu Thr Arg Gly Cys Asp His Ser Thr Glu Lys
 20 25 30
 Gly Ser Glu Lys Pro Val Arg Thr Lys Val Arg His Leu Ala Leu Lys
 35 40 45
 Pro Leu Ser Ala Ile Leu Leu Ser Leu Gly Met Ala Ser Ile Pro Gln
 50 55 60
 Ser Val Leu Ala Ser Gly Leu Gln Gly Met Ser Val Val His Gly Thr
 65 70 75 80

98

Ala	Thr	Met	Gln	Val	Asp	Gly	Asn	Lys	Thr	Thr	Ile	Arg	Asn	Ser	Val	85	90	95
Asn	Ala	Ile	Ile	Asn	Trp	Lys	Gln	Phe	Asn	Ile	Asp	Gln	Asn	Glu	Met	100	105	110
Glu	Gln	Phe	Leu	Gln	Glu	Ser	Ser	Asn	Ser	Ala	Val	Phe	Asn	Arg	Val	115	120	125
Thr	Ser	Asp	Gln	Ile	Ser	Gln	Leu	Lys	Gly	Ile	Leu	Asp	Ser	Asn	Gly	130	135	140
Gln	Val	Phe	Leu	Ile	Asn	Pro	Asn	Gly	Ile	Thr	Ile	Gly	Lys	Asp	Ala	145	150	155
Ile	Ile	Asn	Thr	Asn	Gly	Phe	Thr	Ala	Ser	Thr	Leu	Asp	Ile	Ser	Asn	165	170	175
Glu	Asn	Ile	Lys	Ala	Arg	Asn	Phe	Thr	Leu	Glu	Gln	Thr	Lys	Asp	Lys	180	185	190
Ala	Leu	Ala	Glu	Ile	Val	Asn	His	Gly	Leu	Ile	Thr	Val	Gly	Lys	Asp	195	200	205
Gly	Ser	Val	Asn	Leu	Ile	Gly	Gly	Lys	Val	Lys	Asn	Glu	Gly	Val	Ile	210	215	220
Ser	Val	Asn	Gly	Gly	Ser	Ile	Ser	Leu	Leu	Ala	Gly	Gln	Lys	Ile	Thr	225	230	235
Ile	Ser	Asp	Ile	Ile	Asn	Pro	Thr	Ile	Thr	Tyr	Ser	Ile	Ala	Ala	Pro	245	250	255
Glu	Asn	Glu	Ala	Ile	Asn	Leu	Gly	Asp	Ile	Phe	Ala	Lys	Gly	Gly	Asn	260	265	270
Ile	Asn	Val	Arg	Ala	Ala	Thr	Ile	Arg	Asn	Lys	Gly	Lys	Leu	Ser	Ala	275	280	285
Asp	Ser	Val	Ser	Lys	Asp	Lys	Ser	Gly	Asn	Ile	Val	Leu	Ser	Ala	Lys	290	295	300
Glu	Gly	Glu	Ala	Glu	Ile	Gly	Gly	Val	Ile	Ser	Ala	Gln	Asn	Gln	Gln	305	310	315
Ala	Lys	Gly	Gly	Lys	Leu	Met	Ile	Thr	Gly	Asp	Lys	Val	Thr	Leu	Lys	325	330	335
Thr	Gly	Ala	Val	Ile	Asp	Leu	Ser	Gly	Lys	Glu	Gly	Gly	Glu	Thr	Tyr	340	345	350
Leu	Gly	Gly	Asp	Glu	Arg	Gly	Glu	Gly	Lys	Asn	Gly	Ile	Gln	Leu	Ala	355	360	365
Lys	Lys	Thr	Thr	Leu	Glu	Lys	Gly	Ser	Thr	Ile	Asn	Val	Ser	Gly	Lys	370	375	380
Glu	Lys	Gly	Gly	Arg	Ala	Ile	Val	Trp	Gly	Asp	Ile	Ala	Leu	Ile	Asp	385	390	395
Gly	Asn	Ile	Asn	Ala	Gln	Gly	Ser	Asp	Ile	Ala	Lys	Thr	Gly	Gly	Phe	405	410	415
Val	Glu	Thr	Ser	Gly	His	Asp	Leu	Ser	Ile	Gly	Asp	Asp	Val	Ile	Val	420	425	430

Asp Ala Lys Glu Trp Leu Leu Asp Pro Asp Asp Val Ser Ile Glu Thr
 435 440 445
 Leu Thr Ser Gly Arg Asn Asn Thr Gly Glu Asn Gln Gly Tyr Thr Thr
 450 455 460
 Gly Asp Gly Thr Lys Glu Ser Pro Lys Gly Asn Ser Ile Ser Lys Pro
 465 470 475 480
 Thr Leu Thr Asn Ser Thr Leu Glu Gln Ile Leu Arg Arg Gly Ser Tyr
 485 490 495
 Val Asn Ile Thr Ala Asn Asn Arg Ile Tyr Val Asn Ser Ser Ile Asn
 500 505 510
 Leu Ser Asn Gly Ser Leu Thr Leu His Thr Lys Arg Asp Gly Val Lys
 515 520 525
 Ile Asn Gly Asp Ile Thr Ser Asn Glu Asn Gly Asn Leu Thr Ile Lys
 530 535 540
 Ala Gly Ser Trp Val Asp Val His Lys Asn Ile Thr Leu Gly Thr Gly
 545 550 555 560
 Phe Leu Asn Ile Val Ala Gly Asp Ser Val Ala Phe Glu Arg Glu Gly
 565 570 575
 Asp Lys Ala Arg Asn Ala Thr Asp Ala Gln Ile Thr Ala Gln Gly Thr
 580 585 590
 Ile Thr Val Asn Lys Asp Asp Lys Gln Phe Arg Phe Asn Asn Val Ser
 595 600 605
 Leu Asn Gly Thr Gly Lys Gly Leu Lys Phe Ile Ala Asn Gln Asn Asn
 610 615 620
 Phe Thr His Lys Phe Asp Gly Glu Ile Asn Ile Ser Gly Ile Val Thr
 625 630 635 640
 Ile Asn Gln Thr Thr Lys Lys Asp Val Lys Tyr Trp Asn Ala Ser Lys
 645 650 655
 Asp Ser Tyr Trp Asn Val Ser Ser Leu Thr Leu Asn Thr Val Gln Lys
 660 665 670
 Phe Thr Phe Ile Lys Phe Val Asp Ser Gly Ser Asn Gly Gln Asp Leu
 675 680 685
 Arg Ser Ser Arg Arg Ser Phe Ala Gly Val His Phe Asn Gly Ile Gly
 690 695 700
 Gly Lys Thr Asn Phe Asn Ile Gly Ala Asn Ala Lys Ala Leu Phe Lys
 705 710 715 720
 Leu Lys Pro Asn Ala Ala Thr Asp Pro Lys Lys Glu Leu Pro Ile Thr
 725 730 735
 Phe Asn Ala Asn Ile Thr Ala Thr Gly Asn Ser Asp Ser Ser Val Met
 740 745 750
 Phe Asp Ile His Ala Asn Leu Thr Ser Arg Ala Ala Gly Ile Asn Met
 755 760 765
 Asp Ser Ile Asn Ile Thr Gly Gly Leu Asp Phe Ser Ile Thr Ser His
 770 775 780

100

Asn Arg Asn Ser Asn Ala Phe Glu Ile Lys Lys Asp Leu Thr Ile Asn
 785 790 795 800
 Ala Thr Gly Ser Asn Phe Ser Leu Lys Gln Thr Lys Asp Ser Phe Tyr
 805 810 815
 Asn Glu Tyr Ser Lys His Ala Ile Asn Ser Ser His Asn Leu Thr Ile
 820 825 830
 Leu Gly Gly Asn Val Thr Leu Gly Gly Glu Asn Ser Ser Ser Ile
 835 840 845
 Thr Gly Asn Ile Asn Ile Thr Asn Lys Ala Asn Val Thr Leu Gln Ala
 850 855 860
 Asp Thr Ser Asn Ser Asn Thr Gly Leu Lys Lys Arg Thr Leu Thr Leu
 865 870 875 880
 Gly Asn Ile Ser Val Glu Gly Asn Leu Ser Leu Thr Gly Ala Asn Ala
 885 890 895
 Asn Ile Val Gly Asn Leu Ser Ile Ala Glu Asp Ser Thr Phe Lys Gly
 900 905 910
 Glu Ala Ser Asp Asn Leu Asn Ile Thr Gly Thr Phe Thr Asn Asn Gly
 915 920 925
 Thr Ala Asn Ile Asn Ile Lys Gly Val Val Lys Leu Gly Asp Ile Asn
 930 935 940
 Asn Lys Gly Gly Leu Asn Ile Thr Thr Asn Ala Ser Gly Thr Gln Lys
 945 950 955 960
 Thr Ile Ile Asn Gly Asn Ile Thr Asn Glu Lys Gly Asp Leu Asn Ile
 965 970 975
 Lys Asn Ile Lys Ala Asp Ala Glu Ile Gln Ile Gly Gly Asn Ile Ser
 980 985 990
 Gln Lys Glu Gly Asn Leu Thr Ile Ser Ser Asp Lys Val Asn Ile Thr
 995 1000 1005
 Asn Gln Ile Thr Ile Lys Ala Gly Val Glu Gly Gly Arg Ser Asp Ser
 1010 1015 1020
 Ser Glu Ala Glu Asn Ala Asn Leu Thr Ile Gln Thr Lys Glu Leu Lys
 1025 1030 1035 1040
 Leu Ala Gly Asp Leu Asn Ile Ser Gly Phe Asn Lys Ala Glu Ile Thr
 1045 1050 1055
 Ala Lys Asn Gly Ser Asp Leu Thr Ile Gly Asn Ala Ser Gly Gly Asn
 1060 1065 1070
 Ala Asp Ala Lys Lys Val Thr Phe Asp Lys Val Lys Asp Ser Lys Ile
 1075 1080 1085
 Ser Thr Asp Gly His Asn Val Thr Leu Asn Ser Glu Val Lys Thr Ser
 1090 1095 1100
 Asn Gly Ser Ser Asn Ala Gly Asn Asp Asn Ser Thr Gly Leu Thr Ile
 1105 1110 1115 1120
 Ser Ala Lys Asp Val Thr Val Asn Asn Asn Val Thr Ser His Lys Thr
 1125 1130 1135

101

Ile Asn Ile Ser Ala Ala Ala Gly Asn Val Thr Thr Lys Glu Gly Thr
 1140 1145 1150
 Thr Ile Asn Ala Thr Thr Gly Ser Val Glu Val Thr Ala Gln Asn Gly
 1155 1160 1165
 Thr Ile Lys Gly Asn Ile Thr Ser Gln Asn Val Thr Val Thr Ala Thr
 1170 1175 1180
 Glu Asn Leu Val Thr Thr Glu Asn Ala Val Ile Asn Ala Thr Ser Gly
 1185 1190 1195 1200
 Thr Val Asn Ile Ser Thr Lys Thr Gly Asp Ile Lys Gly Gly Ile Glu
 1205 1210 1215
 Ser Thr Ser Gly Asn Val Asn Ile Thr Ala Ser Gly Asn Thr Leu Lys
 1220 1225 1230
 Val Ser Asn Ile Thr Gly Gln Asp Val Thr Val Thr Ala Asp Ala Gly
 1235 1240 1245
 Ala Leu Thr Thr Thr Ala Gly Ser Thr Ile Ser Ala Thr Thr Gly Asn
 1250 1255 1260
 Ala Asn Ile Thr Thr Lys Thr Gly Asp Ile Asn Gly Lys Val Glu Ser
 1265 1270 1275 1280
 Ser Ser Gly Ser Val Thr Leu Val Ala Thr Gly Ala Thr Leu Ala Val
 1285 1290 1295
 Gly Asn Ile Ser Gly Asn Thr Val Thr Ile Thr Ala Asp Ser Gly Lys
 1300 1305 1310
 Leu Thr Ser Thr Val Gly Ser Thr Ile Asn Gly Thr Asn Ser Val Thr
 1315 1320 1325
 Thr Ser Ser Gln Ser Gly Asp Ile Glu Gly Thr Ile Ser Gly Asn Thr
 1330 1335 1340
 Val Asn Val Thr Ala Ser Thr Gly Asp Leu Thr Ile Gly Asn Ser Ala
 1345 1350 1355 1360
 Lys Val Glu Ala Lys Asn Gly Ala Ala Thr Leu Thr Ala Glu Ser Gly
 1365 1370 1375
 Lys Leu Thr Thr Gln Thr Gly Ser Ser Ile Thr Ser Ser Asn Gly Gln
 1380 1385 1390
 Thr Thr Leu Thr Ala Lys Asp Ser Ser Ile Ala Gly Asn Ile Asn Ala
 1395 1400 1405
 Ala Asn Val Thr Leu Asn Thr Thr Gly Thr Leu Thr Thr Thr Gly Asp
 1410 1415 1420
 Ser Lys Ile Asn Ala Thr Ser Gly Thr Leu Thr Ile Asn Ala Lys Asp
 1425 1430 1435 1440
 Ala Lys Leu Asp Gly Ala Ala Ser Gly Asp Arg Thr Val Val Asn Ala
 1445 1450 1455
 Thr Asn Ala Ser Gly Ser Gly Asn Val Thr Ala Lys Thr Ser Ser Ser
 1460 1465 1470
 Val Asn Ile Thr Gly Asp Leu Asn Thr Ile Asn Gly Leu Asn Ile Ile
 1475 1480 1485

102

Ser Glu Asn Gly Arg Asn Thr Val Arg Leu Arg Gly Lys Glu Ile Asp
 1490 1495 1500
 Val Lys Tyr Ile Gln Pro Gly Val Ala Ser Val Glu Glu Val Ile Glu
 1505 1510 1515 1520
 Ala Lys Arg Val Leu Glu Lys Val Lys Asp Leu Ser Asp Glu Glu Arg
 1525 1530 1535
 Glu Thr Leu Ala Lys Leu Gly Val Ser Ala Val Arg Phe Val Glu Pro
 1540 1545 1550
 Asn Asn Ala Ile Thr Val Asn Thr Gln Asn Glu Phe Thr Thr Lys Pro
 1555 1560 1565
 Ser Ser Gln Val Thr Ile Ser Glu Gly Lys Ala Cys Phe Ser Ser Gly
 1570 1575 1580
 Asn Gly Ala Arg Val Cys Thr Asn Val Ala Asp Asp Gly Gln Gln Pro
 1585 1590 1595 1600

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 29 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Asp Glu Val Ile Glu Ala Lys Arg Ile Leu Glu Lys Val Lys Asp
 1 5 10 15
 Leu Ser Asp Glu Glu Arg Glu Ala Leu Ala Lys Leu Gly
 20 25

CLAIMS

What I claim is:

1. An isolated and purified nucleic acid molecule encoding a high molecular weight protein (HMW) HMW3 or HMW4 of a non-typeable *Haemophilus* strain or a variant or fragment of said protein retaining the immunological ability to protect against disease caused by a non-typeable *Haemophilus* strain, having:
 - (a) the DNA sequence shown in Figure 8 (SEQ ID No: 7) and encoding protein HMW3 having the derived amino acid sequence of Figure 10 (SEQ ID No: 9), or
 - (b) the DNA sequence shown in Figure 9 (SEQ ID No: 8) and encoding protein HMW4 having the derived amino acid sequence of Figure 10 (SEQ ID No: 10).
2. An isolated and purified nucleic acid molecule encoding a high molecular weight protein (HMW) of a non-typeable *Haemophilus* strain, which is selected from the group consisting of:
 - (a) a DNA sequence as shown in any one of Figures 8 and 9 (SEQ ID Nos: 7 and 8);
 - (b) a DNA sequence encoding an amino acid sequence as shown in Figure 10 (SEQ ID Nos: 9 and 10); or
 - (c) a DNA sequence encoding a high molecular weight protein of a non-typeable *Haemophilus* strain which hybridizes under stringent conditions to any one of the DNA sequences of (a) and (b).
3. The nucleic acid molecule of claim 2 wherein the DNA sequence (c) have at least about a 90% identity of sequence to the DNA sequences (a) or (b).
4. A vector for transformation of a host comprising the nucleic acid molecule of claim 2.
5. An isolated and purified high molecular weight (HMW) protein of non-typeable *Haemophilus* or any variant or fragment thereof retaining the immunological ability to protect against disease caused by a non-typeable *Haemophilus* strain, which is characterized by at least

one surface-exposed B-cell epitope which is recognized by monoclonal antibody AD6.

6. The protein of claim 5 which is HMW1 encoded by the DNA sequence shown in Figure 1 (SEQ ID No: 1), having the derived amino acid sequence of Figure 2 (SEQ ID No: 2) and having an apparent molecular weight of 125 kDa.

7. The protein claim 5 which is HMW2 encoded by the DNA sequence shown in Figure 3 (SEQ ID No: 3) and having the derived amino acid sequence of Figure 4 (SEQ ID No: 4) and having an apparent molecular weight of 120 kDa.

8. The protein claimed in claim 5 which is HMW3 encoded by the DNA sequence shown in Figure 8 (SEQ ID No: 7) and having the derived amino acid sequence of Figure 10 (SEQ ID No: 9) and having an apparent molecular weight of 125 kDa.

9. The protein claimed in claim 5 which is HMW4 encoded by the DNA sequence shown in Figure 9 (SEQ ID No: 8) and having the derived amino acid sequence shown in Figure 10 (SEQ ID No: 10) and having an apparent molecular weight of 123 kDa.

10. A conjugate comprising a protein as claimed in claim 5 linked to an antigen, hapten or polysaccharide for eliciting an immune response to said antigen, hapten or polysaccharide.

11. The conjugate as claimed in claim 10 wherein said polysaccharide is a protective polysaccharide against *Haemophilus influenzae* type b.

12. A synthetic peptide having an amino acid sequence containing at least six amino acids and no more than 150 amino acids and corresponding to at least one protective epitope of a high molecular weight protein HMW1, HMW2, HMW3 or HMW4 of non-typeable *Haemophilus influenzae*, wherein the epitope is recognized by at least one of monoclonal antibodies AD6 and 10C5.

13. The peptide as claimed in claim 12 wherein the epitope is located within 75 amino acids of the carboxy terminus of the HMW1 or HMW2 protein.

//73

FIG. 1A. DNA SEQUENCE OF HIGH MOLECULAR WEIGHT PROTEIN

I (HMW1)

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1  ACAGCGTTCT CTTAATACTA GTACAAACCC ACAATAAAAT ATGACAAACA
51  ACAATTACAA CACCTTTTTC GCAGTCTATA TGCAAAATATT TTAAAAAATA
101  GTATAAATCC GCCATATATA ATGGTATAAA CTTTCATCTT TCATCTTTCA
151  TCTTTCATCT TTCATCTTTC ATCTTTCATC TTTCATCTTT CATCTTTCAT
201  CTTTTCATCT TCATCTTTTCA TCTTTCATCT TTCATCTTTC ACATGCCCTG
251  ATGAACCGAG GGAAGGGAGG GAGGGGCAAG AATGAAGAGG GAGCTGAACG
301  AACGCAAAATG ATAAAGTAAT TTAATTGTTC AACTAACCTT AGGAGAAAAA
351  ATGAACAAGC TATATCGTCT CAAATTCAGC AAACGCCCTGA ATGCTTTGGT
401  TGCTGTGTCT GAATTGGCAC GGGGTGTGTA CCATTCCACA GAAAAGGCA
451  GCGAAAAACC TGCTCGCATG AAAGTCCGTC ACTTAGCGTT AAAGCCACTT
501  TCCGCTATGT TACTATCTTT AGGTGTAACA TCTATTCCAC AATCTGTTTT
551  AGCAAGCGGC TTACAAGGAA TGGATGTAGT ACACGGCACA GCCACTATGC
601  AAGTAGATGG TAATAAAACC ATTATCCGCA ACAGTGTGTA CGATATCATT
651  AATTGGAAAC AATTAAACAT CGACCAAAAT GAAATGGTGC AGTTTTTACA
701  AGAAAAACAAC AACTCCGCCG TATTCAACCG TGTTACATCT AACCAAATCT

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RECTIFIED SHEET (RULE 91)

FIG. 1B.

751 CCCAATTAAA AGGATTTTA GATTCTAACG GACAAGTCTT TTTAATCAAC
801 CCAAATGGTA TCACAATAGG TAAAGACGCA ATTATTAAACA CTAATGGCTT
851 TACGGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG GCGCGTAATT
901 TCACCTTCGA GCAAACCAA GATAAGCGC TCGCTGAAAT TGTGAATCAC
951 GGTTTAATTA CTGTCGGTAA AGACGGCAGT GTAAATCTTA TTGGTGGCAA
1001 AGTGAAAAAC GAGGGTGTGA TTAGCGTAAA TGGTGGCAGC ATTCTCTTAC
1051 TCGCAGGGCA AAAATCACC ATCAGCGATA TAATAAACCC AACCATTACT
1101 TACAGCATTG CCGCGCCTGA AAATGAAGCG GTCAATCTGG GCGATATTTT
1151 TGCCAAAGGC GGTAACATTA ATGTCCGTGC TGCCACTATT CGAAACCAAG
1201 GTAAACTTTC TGCTGATTCT GTAAGCAAAG ATAAAAGCGG CAATATTGTT
1251 CTTTCCGCCA AAGAGGGTGA AGCGAAATT GGCGGTGTAA TTTCCGCTCA
1301 AAATCAGCAA GCTAAAGGCG GCAAGCTGAT GATTACAGGC GATAAAGTCA
1351 CATTAAAAAC AGGTGCAGTT ATCGACCTTT CAGGTAAAGA AGGGGGAGAA
1401 ACTTACCTTG GCGGTGACGA GCGCGCGAA GGTA AAAAGG GCATTCAATT
1451 AGCAAAGAAA ACCTCTTTAG AAAAAGGCTC AACCATCAAT GTATCAGGCA
1501 AAGAAAAAGG CGGACGGCT ATTGTGTGG GCGATATTGC GTTAATTGAC

2/73

RECTIFIED SHEET (RULE 91)

3/73

FIG. 1C.

1551 GGCAATATTA ACGCTCAAGG TAGTGGTGAT ATCGCTAAAA CCGTGGTTT
1601 TGTGGAGACG TCGGGGCATG ATTTATTCAT CAAAGACAAAT GCAATTGTG
1651 ACGCCAAAGA GTGGTTGTTA GACCCGGATA ATGTA TCTAT TAATGCAGAA
1701 ACAGCAGGAC GCAGCAATAC TTCAGAAAGAC GATGAATACA CGGGATCCGC
1751 GAATAGTGCC AGCACCCCAA AACGAAACAA AGAAAAGACA ACATTAACAA
1801 ACACAACTCT TGAGAGTATA CTAAAAAAG GTACCTTTGT TAACATCACT
1851 GCTAATCAAC GCATCTATGT CAATAGCTCC ATTAATTTAT CCAATGGCAG
1901 CTTAACTCTT TGGAGTGAGG GTCGGAGCGG TGGCGGCGTT GAGATTAACA
1951 ACGATATTAC CACCGGTGAT GATACCAGAG GTGCAAACTT AACAAATTAC
2001 TCAGGCGGCT GGGTTGATGT TCATAAAAAT ATCTCACTCG GGGCGCAAG
2051 TAACATAAAC ATTACAGCTA AACAAAGATAT CGCCTTTGAG AAAGGAAGCA
2101 ACCAAGTCAT TACAGGTCAA GGGACTATTA CCTCAGGCAA TCAAAAAGGT
2151 TTTAGATTTA ATAATGTCTC TCTAAACGGC ACTGGCAGCG GACTGCAATT
2201 CACCACTAAA AGAACCAATA AATACGCTAT CACAAATAAA TTTGAAGGGA
2251 CTTTAAATAT TTCAGGGAAA GTGAACATCT CAATGGTTTT ACCTAAAAAT
2301 GAAAGTGGAT ATGATAAATT CAAAGGACGC ACTTACTGGA ATTTAACCTC

RECTIFIED SHEET (RULE 91)

4/73

FIG. 1D.

2351 CTTAAATGTT TCCGAGAGTG GCGAGTTTAA CCTCACTATT GACTCCAGAG
2401 GAAGCGATAG TGCAGGCACA CTTACCCAGC CTTATAAATT AAACGGTATA
2451 TCATTCAACA AAGACACTAC CTTTAAATGTT GAACGAAATG CAAGAGTCAA
2501 CTTTGACATC AAGGCACCAA TAGGATATAA TAAGTATTCT AGTTGAATT
2551 ACGCATCATT TAATGGAAAC ATTCAGTTT CGGGAGGGG GAGTGTGAT
2601 TTCACACTTC TCGCCTCATC CTCTAACGTC CAAACCCCG GTGTAGTTAT
2651 AAATTCTAAA TACTTTAATG TTTC AACAGTTA AGATT'AAAA
2701 CTTCAGGCTC AACAAAACCT GGCTTCTCAA TAGAGAAAGA TTTAACTTTA
2751 AATGCCACCG GAGGCAACAT AACACTTTTG CAAGTTGAAG GCACCGATGG
2801 AATGATTGGT AAAGGCATTG TAGCCAAAAA AAACATAACC TTTGAAGGAG
2851 GTAACATCAC CTTTGGCTCC AGGAAAGCCG TAACAGAAAT CGAAGGCAAT
2901 GTTACTATCA ATAACAACGC TAACGTCACT CTTATCGGTT CGGATTTTGA
2951 CAACCATCAA AAACCTTTAA CTATTAAAA AGATGTCATC ATTAAATAGCG
3001 GCAACCTTAC CGCTGGAGGC AATATTGTCA ATATAGCCGG AAATCTTACC
3051 GTTGAAAGTA ACGCTAATTT CAAAGCTATC ACAAATTCA CTTTAAATGT
3101 AGGCGGCTTG TTGACAACA AAGCAATTC AAATATTTC ATTGCCAAAG
3151 GAGGGGCTCG CTTTAAAGAC ATTGATAATT CCAAGAAATT AGCATCACC

RECTIFIED SHEET (RULE 91)

FIG. 1E.

3201 ACCAACTCCA GCTCCACTTA CCGCACTATT ATAAGCGGCA ATATAACCAA
3251 TAAAAACGGT GATTAAATA TTACGAACGA AGGTAGTGAT ACTGAAATGC
3301 AAATTGGCGG CGATGTCTCG CAAAAGAAG GTAATCTCAC GATTCTTCT
3351 GACAAAATCA ATATTACCAA ACAGATAACA ATCAAGGCAG GTGTTGATGG
3401 GGAGAAATCC GATTCAGACG CGACAAACAA TGCCAATCTA ACCATTAAAA
3451 CCAAAGAATT GAAATTAACG CAAGACCTAA ATATTTTCAGG TTTCAATAAA
3501 GCAGAGATTA CAGCTAAAGA TGGTAGTGAT TTAACATATTG GTAACACCAA
3551 TAGTGCTGAT GGTACTAATG CCAAAAAAGT AACCTTTAAC CAGGTTAAAG
3601 ATTCAAAAAT CTCTGCTGAC GGTCAACAAG TGACACTACA CAGCAAAGTG
3651 GAAACATCCG GTAGTAATAA CAACACTGAA GATAGCAGTG ACAATAATGC
3701 CGGCTTAACT ATCGATGCAA AAAATGTAAC AGTAAACAAC AATATTACTT
3751 CTCACAAAGC AGTGAGCATC TCTGCCACAA GTGGAGAAAT TACCACTAAA
3801 ACAGGTACAA CCATTAAACG AACCACTGGT AACGTGGAGA TAACCGCTCA
3851 AACAGGTAGT ATCCTAGGTG GAATTGAGTC CAGCTCTGGC TCTGTAACAC
3901 TTAGTGCAAC CGAGGGCGCT CTTGCTGTAA GCAATATTTC GGGCAACACC
3951 GTTACTGTTA CTGCAAAATAG CGGTGCATTA ACCACTTTGG CAGGCTCTAC

57/73

RECTIFIED SHEET (RULE 91)

6/73

FIG. 1F.

4001 AATTAAAGGA ACCGAGAGTG TAACCACTTC AAGTCAATCA GCGGATATCG
4051 GCGGTACGAT TTCTGGTGGC ACAGTAGAGG TTAAAGCAAC CGAAAGTTTA
4101 ACCACTCAAT CCAATTCAAA AATTAAAGCA ACAACAGGCG AGGCTAACGT
4151 AACAAAGTGCA ACAGGTACAA TTGGTGGTAC GATTCCGGT AATACGGTAA
4201 ATGTTACGGC AAACGCTGGC GATTTAACAG TTGGGAATGG CGCAGAAATT
4251 AATGCGACAG AAGGAGCTGC AACCTTAACT ACATCATCGG GCAAATTAAAC
4301 TACCGAAGCT AGTTCAACACA TTACTTCAGC CAAGGTCAG GTAAATCTTT
4351 CAGCTCAGGA TGGTAGCGTT GCAGGAAGTA TTAATGCCGC CAATGTGACA
4401 CTAAATACTA CAGGCACTTT AACTACCGTG AAGGGTTCAA ACATTAATGC
4451 AACCAGCGGT ACCTTGTTA TTAACGCAA AGACGCTGAG CTAAATGGCG
4501 CAGCATTTGG TAACCCACACA GTGGTAAATG CAACCAACGC AAATGGCTCC
4551 GGCAGCGTAA TCGCGACAAC CTCAAGCAGA GTGAACATCA CTGGGGATTT
4601 AATCACAAATA AATGGATTAA ATATCATTTT AAAAAACGGT ATAAACACCCG
4651 TACTGTTAAA AGGCGTTAAA ATTGATGTGA AATACATTCA ACCGGGTATA
4701 GCAAGCGTAG ATGAAGTAAT TGAAGCGAA CGCATCCTTG AGAAGGTAAA
4751 AGATTATCT GATGAAGAAA GAGAAGCGTT AGCTAAACTT GGAGTAAGTG
4801 CTGTACGTTT TATTGAGCCA AATAATACAA TTACAGTCGA TACACAAAAT

RECTIFIED SHEET (RULE 91)

7/23

FIG. 1G.

4851 GAATTTGCAA CCAGACCATTT AAGTCGAATA GTGATTTCTG AAGCAGGGC
4901 GTGTTTCTCA AACAGTGATG GCGCGACGGT GTGCCGTTAAT ATCGCTGATA
4951 ACGGGCGGTA GCGGTCAGTA ATTGACAAGG TAGATTTTCAT CCTGCAATGA
5001 AGTCATTTTA TTTTCGTATT ATTTACTGTG TGGGTTAAAG TTCAGTACCG
5051 GC'TTTACCCA TCTTGTA AAA AATTACGGAG AATACAATAA AGTATTTTTA
5101 ACAGGTTATT ATTATG

RECTIFIED SHEET (RULE 91)

FIG. 2A. AMINO ACID SEQUENCE OF HIGH MOLECULAR WEIGHT

PROTEIN I

1 MNKIYRLKFS KRLNALVAVS ELARGCDHST EKGSEKPARM KVRHLALKPL
51 SALLSLGVT SIPQSVLASG LQMDVVHGT ATMQVDGNKT IIRNSVDAL I
101 NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNQGVFLIN
151 PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTFEQTK DKALAEIVNH
201 GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
251 YSIAAPENEA VNLGDIFAKG GNINVRAATI RNQKLSADS VSKDKSGNIV
301 LSAKEGEAEI GGVisAQNQQ AKGKGLMITG DKVTLKTGAV IDLSGKEGGE
351 TYLGGDERGE GKNGIQLAKK TSLEKKGSTIN VSGKEKGGRA IVWGDIALID
401 GNINAQSGD IAKTGGFVET SGHDLFIKDN AIVDAKEWLI DFDNVSINAE
451 TAGRSNTSED DEYTGSGNSA STPKRNKEKT TLTNNTLESI LKKGTFVNIT
501 ANQRIYVNSS INLSNGSLTL WSEGRSGGV EINNDITTGD DTRGANLTIY
551 SGGWVDVHKN ISLGAQGNIN ITAKQDIAFE KGSNQVITGQ GTITSGNQKG
601 FRFNNVSLNG TGSGLQFTTK RTNKYAITNK FEGTLNISGK VNISMVLPKN
651 ESGYDKFKGR TYWNLTSLNV SESGEFNLT I DSRGSDSAGT LTQPYNLNGI
701 SFNKDTTFNV ERNARVNFDI KAPIGINKYS SLNYASFNGN ISVSGGGSVD

8/73

RECTIFIED SHEET (RULE 91)

9/73

FIG. 2B.

751 FTLLASSNV QTPGVVINSK YFNVSTGSSL RFKTSGSTKT GFSIEKDLTL
 801 NATGGNITLL QVEGTDMIG KGIVAKKNIT FEGGNITFGS RKAVTEIEGN
 851 VTINNANVT LIGSDFDNHQ KPLTIKKDVI INSGNLTAGG NIVNIAGNLT
 901 VESNANFKAI TNFTFNVGGL FDNKGNISNIS IAKGGARFKD IDNSKNLSIT
 951 TNSSSTYRTI ISGNITNKNG DLNITNEGSD TEMQIGGDVS QKEGNLTSS
 1001 DKINITKQIT IKAGVDGENS DSDATNNANL TIKTKELKLT QDLNISGFNK
 1051 AEITAKDGSD LTIGNTNSAD GTNAKKVTFN QVKDSKISAD GHKVTLHSKV
 1101 ETSGSNNNTE DSSDNNAGLT IDAKNVTVNN NITSHKAVSI SATSGEITTK
 1151 TGTINATTG NVEITAQTS ILGGIESSG SVTLTATEGA LAVSNISGNT
 1201 VTVTANS GAL TTAGSTIKG TESVTTSSQS GDIGGTISGG TVEVKATESL
 1251 TTQSNSKIK A TTGEANVTSA TGTIGGTISG NTVNVTANAG DLTVGNGAEI
 1301 NATEGAATLT TSSGKLTTTEA SSHITSAKQ VNLSAQDGSV AGSINAANVT
 1351 LNTTGTLTTV KGSNINATSG TLVINAKDAE LINGAALGNHT VVNATNANGS
 1401 GSVIATTSSR VNITGDLITI NGLNIISKNG INTVLLKGVK IDVKYIQPGI
 1451 ASVDEVIEAK RILEKVKDLS DEEREALAKL GVSAVRFIEP NNTITVDTQN
 1501 EFATRPLSRI VISEGRACFS NSDGATVCVN IADNGR

RECTIFIED SHEET (RULE 91)

FIG. 3A.

DNA SEQUENCE OF HIGH MOLECULAR WEIGHT
PROTEIN II (HMW2)

1 TAAATATACA AGATAATAAA AATAAATCAA GATTTTGTG ATGACAAACA
51 ACAATTACAA CACCTTTTTT GCAGCTATA TGCAAATATT TTAAAAAAT
101 AGTATAAATC CGCCATATAA AATGGTATAA TCTTTCATCT TTCATCTTTA
151 ATCTTTTCATC TTTTCATCTTT CATCTTTCAT CTTTCATCTT TCATCTTTCA
201 TCTTTTCATCT TTCATCTTTC ATCTTTTCATC TTTTCATCTTT CACATGAAAT
251 GATGAACCGA GGAAGGGAG GGAGGGGCAA GAATGAAGAG GGAGCTGAAC
301 GAACGCAAAT GATAAAGTAA TTTAATTGTT CAACTAACCT TAGGAGAAAA
351 TATGAACAAG ATATATCGTC TCAAATTCAG CAAACGCCCTG AATGCTTTGG
401 TTGCTGTGTC TGAATTGGCA CGGGGTTGTG ACCATTCCAC AGAAAAAGGC
451 TTCCGCTATG TTAATATCTT TAGGTGTAAC CACTTAGCGT TAAAGCCACT
501 TTCCGCTATG TTAATATCTT TAGGTGTAAC ATCTATTCCA CAATCTGTTT
551 TAGCAAGCGG CTTACAAGGA ATGGATGTAG TACACGGCAC AGCCACTATG
601 CAAGTAGATG GTAAATAAAC CATTATCCGC AACAGTGTG ACGCTATCAT
651 TAATTGGAAA CAATTTAACA TCGACCAAAA TGAAATGGTG CAGTTTTTAC
701 AAGAAACAA CAACTCCGCC GTATTCAACC GTGTTACATC TAACCAAAATC

10/73

RECTIFIED SHEET (RULE 91)

FIG. 3B.

751 TCCCAATTAA AAGGATTTT AGATTCTAAC GGACAAGTCT TTTTAATCAA
801 CCCAAATGGT ATCACAATAG GTAAAGACGC AATTATTAAC ACTAATGGCT
851 TTACGGCTTC TACGCTAGAC ATTTCTAACG AAAACATCAA GCGCGTAAT
901 TTCACCTTCG AGCAAACCAA AGATAAAGCG CTCGCTGAAA TTGTGAATCA
951 CGGTTTAATT ACTGTCGGTA AAGACGGCAG TGTAATCTT ATTGGTGGCA
1001 AAGTGAAAAA CGAGGGTGTG ATTAGCGTAA ATGGTGGCAG CATTCTTTA
1051 CTCGCAGGGC AAAAAATCAC CATCAGCGAT ATAATAAACC CAACCATTA
1101 TTACAGCATT GCCGCGCCTG AAAATGAAGC GGTCAATCTG GCGATATTT
1151 TTGCCAAAGG CGGTAACATT AATGTCCGTG CTGCCACTAT TCGAAACCAA
1201 GGTAACCTT CTGCTGATTC TGTAAGCAAA GATAAAAGCG GCAATATTGT
1251 TCTTTCCGCC AAAGAGGGTG AAGCGGAAAT TGGCGGTGTA ATTTCCGCTC
1301 AAAATCAGCA AGCTAAAGGC GGCAAGCTGA TGATTACAGG CGATAAAGTC
1351 ACATTAAAAA CAGGTGCAGT TATCGACCTT TCAGGTAAAG AAGGGGAGA
1401 AACTTACCTT GCGCGTGACG AGCGCGGCGA AGGTAAAAAC GGCATTCAAT
1451 TAGCAAAGAA AACCTCTTTA GAAAAAGGCT CAACCATCAA TGTATCAGGC
1501 AAAGAAAAAG GCGACGCGC TATTGTGTG GCGGATATTG CGTTAATTGA

11/23

RECTIFIED SHEET (RULE 91)

FIG. 3C.

1551 CGGCAATATT AACGCTCAAG GTAGTGTGA TATCGCTAAA ACCGGTGGTT
1601 TTGTGGAGAC ATCGGGGCAT TATTATCCA TTGACAGCAA TGCAATIGTT
1651 AAAACAAAAG AGTGGTGTGCT AGACCCGTGAT GATGTAACAA TTGAAGCCGA
1701 AGACCCCTT CGCAATAATA CCGGTATAAA TGATGAATC CCAACAGGCA
1751 CCGGTGAAGC AAGCGACCCT AAAAAAATA GCGAACTCAA AACACGCTA
1801 ACCAATACAA CTATTTCAAATTATCTGAAA AACGCCCTGGA CAATGAATAT
1851 AACGGCATCA AGAAACTTA CCGTTAATAG CTCAATCAAC ATCGGAAGCA
1901 ACTCCCACCTT AATTCTCCAT AGTAAAGGTC AGCGTGGCGG AGGCGTTCAG
1951 ATTGATGGAG ATATTACTTC TAAAGGCGGA AATTTAACCA TTTATTCTGG
2001 CGGATGGGTT GATGTTTATA AAAATATTAC GCTTGATCAG GGTTTTTTAA
2051 ATATTACCGC CGCTTCCGTA GCTTTTGAAG GTGGAAATAA CAAAGCACGC
2101 GACGGGGCAA ATGCTAAAAT TGTCGCCAG GGCACGTAA CCATTACAGG
2151 AGAGGGAAAA GATTTCAGGG CTAACAACGT ATCTTTAAAC GGAACGGGTA
2201 AAGGCTTGAA TATCATTTCA TCAGTGAATA ATTTAACCCA CAATCTTAGT
2251 GGCACAATTA ACATATCTGG GAATATAACA ATTAACCCAAA CTACGAGAAA
2301 GAACACCTCG TATTGGCAA CCAGCCATGA TTCGCACTGG AACGTCAGTG
2351 CTCTTAATCT AGAGACAGGC GCAAATTTTA CCTTTATTAA ATACATTTCA

12/73

RECTIFIED SHEET (RULE 91)

13/73

FIG. 3D.

2401 AGCAATAGCA AAGGCTTAAC AACACAGTAT AGAAGCTCTG CAGGGGTGAA
 2451 TTTTAACGGC GTAAATGGCA ACATGTCAAT CAATCTCAA GAAGGAGCGA
 2501 AAGTTAATTT CAAATTAAAA CCAAACGAGA ACATGAACAC AAGCAAACCT
 2551 TTACCAATTC GGTTTT TAGC CAATATCACA GCCACTGGTG GGGGCTCTGT
 2601 TTTT TTTGAT ATATATGCCA ACCATTCTGG CAGAGGGGCT GAGTTAAAAA
 2651 TGAGTGAAAT TAAATATCTCT AACGGCGCTA ATTTTACCTT AAATTCCCAT
 2701 GTTCGGGCG ATGACGCCTT TAAAATCAAC AAAGACTTAA CCATAAATGC
 2751 AACCAATTCA AATTTCAGCC TCAGACAGAC GAAAGATGAT TTTTATGACG
 2801 GTACGCACG CAATGCCATC AATTCAACCT ACAACATATC CATCTGGGC
 2851 GGTAATGTCA CCTTGGTGG ACAA AACTCA AGCAGCAGCA TTACGGGGAA
 2901 TATTACTATC GAGAAAGCAG CAAATGTTAC GCTAGAAGCC AATAACGCCC
 2951 CTAATCAGCA AAACATAAGG GATAGAGTTA TAAA AACTTGG CAGCTTGCTC
 3001 GTTAATGGGA GTTTAAGTTT AACTGGCGAA AATGCAGATA TTAAAGGCAA
 3051 TCTCACTATT TCAGAAAGCG CCACTTTTAA AGGAAAGACT AGAGATACCC
 3101 TAAATATCAC CGGCAATTTT ACCAATAATG GCACTGCCGA AATTAATAA
 3151 ACACAAGGAG TGGTAAA AACT TGGCAATGTT ACCAATGATG GTGATTTAAA

RECTIFIED SHEET (RULE 91)

FIG. 3E.

3201 CATTACCACT CACGCTAAAC GCAACCAAAG AAGCATCATC GCGGAGATA
 3251 TAATCAACAA AAAAGGAAGC TTAAATATTA CAGACAGTAA TAATGATGCT
 3301 GAAATCCAAA TTGGCGGCAA TATCTCGCAA AAAGAAGGCA ACCTCACGAT
 3351 TTCTTCCGAT AAAATTAATA TCACCAAACA GATAACAATC AAAAAGGGTA
 3401 TTGATGGAGA GGACTCTAGT TCAGATGCCA CAAGTAATGC CAACCTAACT
 3451 ATTAAAACCA AAGAATTGAA ATTGACAGAA GACCTAAGTA TTTCAGGTTT
 3501 CAATAAAGCA GAGATTACAG CCAAAGATGG TAGAGATTTA ACTATTGGCA
 3551 ACAGTAATGA CGGTAACAGC GGTGCCGAAG CCAAAACAGT AACTTTTAAC
 3601 AATGTTAAAG ATTCAAAAAT CTCTGCTGAC GGT'CACAATG TGACACTAAA
 3651 TAGCAAAGTG AAAACATCTA GCAGCAATGG CGGACGTGAA AGCAATAGCG
 3701 ACAACGATAC CGGCTTAACT ATTACTGCCA AAAATGTAGA AGTAAACAAA
 3751 GATAATTACTT CTCTCAAAC AGTAAATATC ACCGCGTCGG AAAAGGTAC
 3801 CACCACAGCA GGCTCGACCA TTAACGCAAC AAATGGCAA GCAAGTATTA
 3851 CAACCAAAC AGGTGATATC AGCGGTACGA TT'TCCGGTAA CACGGTAAGT
 3901 GTTAGCGCGA CTGGTGATTT AACCACTAAA TCCGGCTCAA AAATTGAAGC
 3951 GAAATCGGGT GAGGCTAATG TAACAAGTGC AACAGGTACA ATTGGCGGTA

14
 13

RECTIFIED SHEET (RULE 91)

FIG. 3F.

4001 CAATTTCCGG TAATACGGTA AATGTTACGG CAAACGCTGG CGATTTAACA
4051 GTTGGGAATG GCGCAGAAAT TAATGCGACA GAAGGAGCTG CAACCTTAAC
4101 CGCAACAGGG AATACCTTGA CTAAGTGAAGC CGGTCTTAGC ATCACTTCAA
4151 CTAAGGGTCA GGTAGACCTC TTGGCTCAGA ATGGTAGCAT CGCAGGAAGC
4201 ATTAATGCTG CTAATGTGAC ATTAATACT ACAGGCACCT TAACCACCGT
4251 GGCAGGCTCG GATAATTAAG CAACCAGCGG CACCTTGGTT ATTAACGCAA
4301 AAGATGCTAA GCTAAATGGT GATGCATCAG GTGATAGTAC AGAAGTGAAT
4351 GCAGTCAACG CAAGCGGCTC TGGTAGTGTG ACTGCGGCAA CCTCAAGCAG
4401 TGTGAATATC ACTGGGGATT TAAACACAGT AAATGGGTTA AATATCATTT
4451 CGAAAGATGG TAGAAACACT GTGCGCTTAA GAGGCAAGGA AATTGAGGTG
4501 AAATATATCC AGCCAGGTGT AGCAAGTGTA GAAGAAGTAA TTGAAGCGAA
4551 ACGCGTCCTT GAAAAGTAA AAGATTTATC TGATGAAGAA AGAGAAACAT
4601 TAGCTAAACT TGGTGTAAGT GCTGTACGTT TTGTTGAGCC AAATAATACA
4651 ATTACAGTCA ATACACAAA TGAATTTACA ACCAGACCGT CAAGTCAAGT
4701 GATAATTCT GAAGGTAAGG CGTGTTTCTC AAGTGTAAAT GGCGCACGAG
4751 TATGTACCAA TGTGCTGAC GATGGACAGC CGTAGTCAGT AATTGACAAG
4801 GTAGATTCA TCCTGCAATG AAGTCATTTT ATTTTCGTAT TATTACTGT

15/73

RECTIFIED SHEET (RULE 91)

16/73

FIG. 3G.

4851 GTGGGTAAA GTTCAGTACG GGCTTTACCC ATCTTGTAAG AAATTACGGA
4901 GAATACAATA AAGTATTTT AACAGGTAT TATTATG

RECTIFIED SHEET (RULE 91)

FIG. 4A. AMINO ACID SEQUENCE OF HIGH MOLECULAR WEIGHT

PROTEIN 2

1 MNKIYRLKFS KRLNALVAVS ELARGCDHST EKGSEKPARM KVRHLALKPL
51 SALLSLGVT SIPQSVLASG LQMDVVHGT ATMQVDGNKT IIRNSVDAIL
101 NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNQGVFLIN
151 PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTFEQTK DKALAEIVNH
201 GLITVGKDG S VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
251 YSIAAPENEA VNLGDIFAKG GNINVRAATI RNQKLSADS VSKDKSGNIV
301 LSAKEGEAEI GGVIS AQNQQ AKGKLMITG DKVTLKTGAV IDLSGKEGGE
351 TYLGGDERGE GKNGIQLAKK TSLEKGSTIN VSGKEKGGRA IVWGDIALID
401 GNINAQSGD IAKTGGFVET SGHDLFIKDN AIVDAKEWLL DFDNVSINAE
451 DPLRNTGIN DEFPTGTGEA SDPKKNSELK TTLTNTTISN YLKNAWTMNI
501 TASRKLTVNS SINIGSNSHL ILHSGQRRG GVQIDGDITS KGGNLTIVSG
551 GWVDVHKNIT LDQGFLNITA ASVAFEGGNN KARDAANAKI VAQGTVTITG
601 EGKDFRANNV SLNGTGKGLN IISVVNNLTH NLSGTINISG NITINQTRK
651 NTSYWQTS HD SHWNVSALNL ETGANFTFIK YISSNSKGLT TQYRSSAGVN
701 FNGVNGNMSF NLKEGAKVNF KLKPNENMNT SKPLPIRFLA NITATGGGSV

17/73

RECTIFIED SHEET (RULE 91)

18/23

FIG. 4B.

751 FFDIYANHSG RGAELKMSEI NISNGANFTL NSHVRGDDAF KINKDLTINA
 801 TNSNFSLRQT KDDFYDGYAR NAINSTYNIS ILGGNVTLGG QNSSSITGN
 851 ITIEKAANVT LEANNAPNQO NIRDVVIKLG SLLVNGSLSL TGENADIKGN
 901 LTISESATEFK GKTRDTLNT GNFTNNGTAE INITQGVVKL GNVTN DGDLN
 951 ITTHAKRNQR SIIGGDIINK KGS LNITDSN NDAEIQIGGN ISQKEGNLTI
 1001 SSDKINITKQ ITIKKGIDGE DSSSDAT'SNA NLTIKTKELK LTEDLSISGF
 1051 NKAELITAKDG RDLTIGNSND GNSGAEAKTV TFNNVVKDSKI SADGHNVTNLN
 1101 SKVKTSSSNG GRESNSDNDT GLTITAKNVE VNKDITSLKT VNITASEKVT
 1151 TTAGSTINAT NGKASITTKT GDISGTISGN TVSVSATVDL TTKSGSKIEA
 1201 KSGEANVTSA TGTIGGTISG NTVNVTANAG DLTVGNGAEI NATEGAATLT
 1251 ATGNTLTTEA GSSITSTKGQ VDLLAQNGSI AGSINAANVT LNTTGTLTTV
 1301 AGSDIKATSG TLVINAKDAK LNGDASGDST EVNAVNASGS GSVTAATSSS
 1351 VNITGDLNTV NGLNIISKDG RNTVRLRGKE IEVKYIQPGV ASVEEVIEAK
 1401 RVLEKVKDLS DEERETLAKL GVS AVRFVEP NNTITVNTQN EFTTRPSSQV
 1451 IISEGKACFS SNGARVCTN VADGGQP

RECTIFIED SHEET (RULE 91)

19/73

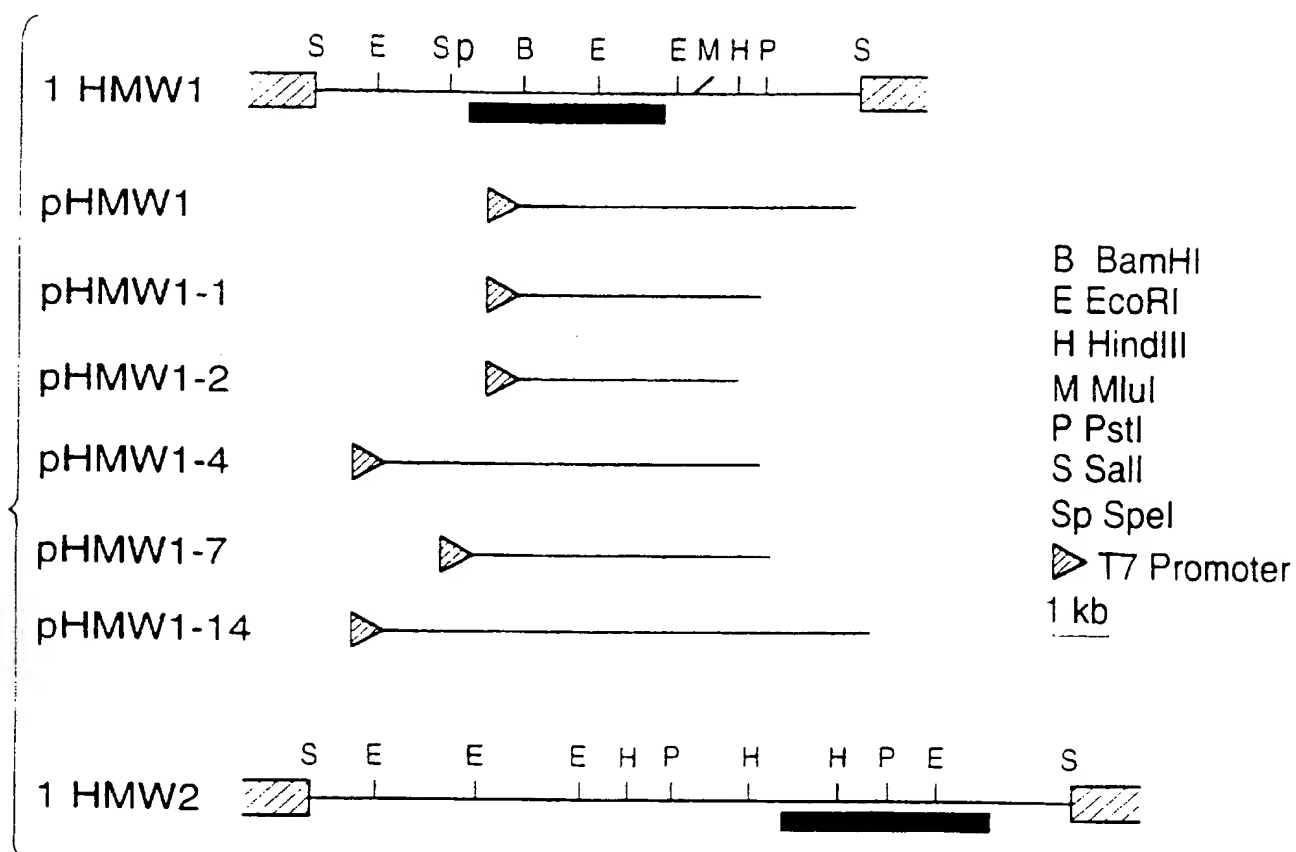
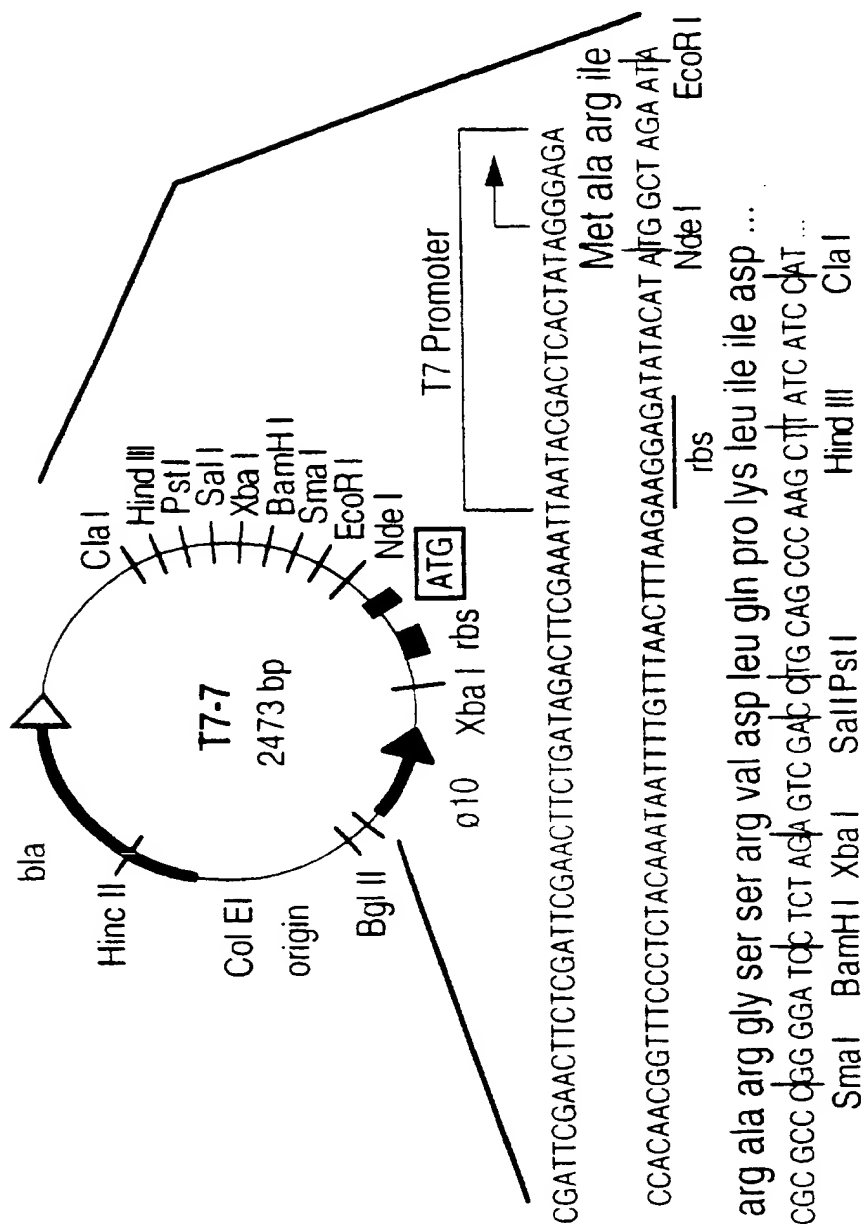


FIG.5 A.

**FIG. 5B.**

(A) Partial restriction maps of representative HMW1 and HMW2 recombinant phage and of HMW1 plasmid subclones. The shaded boxes indicate the locations of the structural genes. In the recombinant phage, transcription proceeds from left to right for the HMW1 gene and from right to left for the HMW2 gene. The methods used for construction of the plasmids shown are described in the text. (B) Restriction map of the T7 expression vector pT7-7. This vector contains the T7 RNA polymerase promoter $\phi 10$, a ribosome - binding site (rbs), and the translational start site for the T7 gene 10 protein upstream from a multiple cloning site (37).

21/73

FIG. 6A.

1 ACAGCGTTCT CTTAATACTA GTACAAACCC ACAATAAAT ATGACAAACA
51 ACAATTACAA CACCTTTTTT GCAGTCTATA TGCAAAATATT TTAAAAAATA
101 GTATAAATCC GCCATATAAA ATGGTATAAT CTTTCATCTT TCATCTTTCA
151 TCTTTTCATCT TTCAATCTTTC ATCTTTTCATC TTTTCATCTTT CATCTTTTCAT
201 CTTTTCATCTT TCATCTTTCA TCTTTTCATCT TTCATCTTTC ACATGAAATG
251 ATGAACCGAG GGAAGGAGG GAGGGCAAG AATGAAGAGG GAGCTGAACG
301 AACGCAAATG ATAAAGTAAT TTAAATTGTTT AACTAACCTT AGGAGAAAAT
351 ATGAACAAGA TATATCGTCT CAAATTCAGC AAACGCCCTGA ATGCTTTGGT
401 TGCTGTGTCT GAATTGGCAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA
451 GCGAAAAACC TGCTCGCATG AAAGTGCCTC ACTTAGCGTT AAAGCCACTT
501 TCCGCTATGT TACTATCTTT AGGTGTAACA TCTATTCAC AATCTGTTTT
551 AGCAAGCGGC TTACAAGGAA TGGATGTAGT ACACGGCACA GCCACTATGC
601 AAGTAGATGG TAATAAAACC ATTATCCGCA ACAGTGTGA CGCTATCATT
651 AATTGGAAAC AATTAAACAT CGACCAAAAT GAAATGGTGC AGTTTTTACA
701 AGAAAACAAC AACTCCGCCG TATTCAACCG TGTTACATCT AACCAAATCT
751 CCCAATTAAA AGGGATTTTA GATTCTAACG GACAAGTCTT TTTAATCAAC

RECTIFIED SHEET (RULE 91)

22/73

FIG. 6B.

801 CCAAATGGTA TCACAAATAGG TAAAGACGCA ATTATTAAACA CTAATGGCTT
851 TACGGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG GCGCGTAATT
901 TCACCTTCGA GCAAACCAA GATAAGCGC TCGCTGAAAT TGTGAATCAC
951 GGTTTAATTA CTGTCGGTAA AGACGGCAGT GTAAATCTTA TTGGTGGCAA
1001 AGTGAAAAC GAGGTGTGA TTAGCGTAA TGGTGGCAGC ATTTCTTTAC
1051 TCGCAGGGCA AAAAATCACC ATCAGCGATA TAATAAACCC AACCATTA
1101 TACAGCATTG CCGCGCCTGA AAATGAAGCG GTCAATCTGG GCGATATTTT
1151 TGCCAAAGGC GGTAACATTA ATGTCCGTGC TGCCACTATT CGAAACCAAG
1251 CTTTCCGCCA AAGAGGGTGA AGCGGAAATT GGCGGTGTAA TTTCCGCTCA
1301 AAATCAGCAA GCTAAAGCG GCAAGCTGAT GATTACAGGC GATAAAGTCA
1351 CATTAAAAC AGGTGCAGTT ATCGACCTTT CAGGTAAAGA AGGGGAGAA
1401 ACTTACCTTG GCGGTGACGA GCGCGCGGAA GGTA AAAACG GCATTCAATT
1451 AGCAAAGAAA ACCTCTTTAG AAAAAGGCTC AACCATCAAT GTATCAGGCA
1501 AAGAAAAAGG CGGACCGGCT ATTGTGTGGG GCGATATTGC GTTAATTGAC
1551 GGCAATATTA ACGCTCAAGG TAGTGTGAT ATCGCTAAAA CCGGTGGTTT
1601 TGTGGAGACG TCGGGGCATG ATTTATTCAT CAAAGACAAT GCAATTGTG

RECTIFIED SHEET (RULE 91)

23/73

FIG. 6C.

1651 ACGCCAAAGA GTGGTTGTTA GACCCGGATA ATGTATCTAT TAATGCAGAA
 1701 ACAGCAGGAC GCAGCAATAC TTCAGAAGAC GATGAATACA CGGGATCCGG
 1751 GAATAGTGCC AGCACCCCAA AACGAAACAA AGAAAAGACA ACATTAAACA
 1801 ACACAACTCT TGAGAGTATA CTAACAAAAG GTACCTTTGT TAACATCACT
 1851 GCTAATCAAC GCATCTATGT CAATAGCTCC ATTAATTTAT CCAATGGCAG
 1901 CTTAACTCTT TGGAGTGAGG GTCGGAGCGG TGGCGGCGTT GAGATTAAACA
 1951 ACGATATTAC CACCGGTGAT GATACCAGAG GTGCCAACTT AACAAATTAC
 2001 TCAGGCGGCT GGGTTGATGT TCATAAAAAT ATCTCACTCG GGGCGCAAGG
 2051 TAACATAAAC ATTACAGCTA AACAAAGATAT CGCCTTTGAG AAAGGAAGCA
 2101 ACCAAGTCAT TACAGGTCAA GGGACTATTA CCTCAGGCAA TCAAAAAGGT
 2151 TTTAGATTTA ATAATGTCTC TCTAAACGGC ACTGGCAGCG GACTGCAATT
 2201 CACCACTAAA AGAACCAATA AATACGCTAT CACAAATAAA TTTGAAGGGA
 2251 CTTTAAATAT TTCAGGGAAA GTGAACATCT CAATGGTTTT ACCTAAAAAT
 2301 GAAAGTGGAT ATGATAAATT CAAAGGACGC ACTTACTGGA ATTTAACCTC
 2351 GAAAGTGGAT ATGATAAATT CAAAGGACGC CCTCACTATT GACTCCAGAG
 2401 GAAGCGATAG TGCAGGCACA CTTACCCAGC CTTATAATTT AACGGTATA
 2451 TCATTCAACA AAGACACTAC CTTTAATGTT GAACGAAATG CAAGAGTCAA

RECTIFIED SHEET (RULE 91)

24/73

FIG. 6D.

2501 CTTTGACATC AAGGCACCAA TAGGGATAAA TAAGTATTCT AGTTTGAATT
2551 ACGCATCATT TAATGGAAC ATTTCAAGTT CCGGAGGGG GAGTGTGAT
2601 TTCACACTTC TCGCCTCATC CTCTAACGTC CAAACCCCG GTGTAGTTAT
2651 AAATTCTAAA TACTTTAATG TTTCAACAGG GTCAAAGTTA AGATTTAAAA
2701 CTCAGGCTC AACAAAACT GGCTTCTCAA TAGAGAAAGA TTAACTTTA
2751 AATGCCACCG GAGGCAACAT AACACTTTG CAAGTTGAAG GCACCGATGG
2801 AATGATTGGT AAAGGCATTG TAGCCAAAAA AACATAACC TT'TGAAGGAG
2851 GTAAGATGAG GTTTGGCTCC AGGAAAGCCG TAACAGAAAT CGAAGGCAAT
2901 GTTACTATCA ATAACAACGC TAACGTCACT CTTATCGGTT CGGATTTTGA
2951 CAACCATCAA AAACCTTTAA CTATTAAAAA AGATGTCATC ATTAATAGCG
3001 GCAACCTTAC CGCTGGAGGC AATATTGTCA ATATAGCCGG AAATCTTACC
3051 GTTGAAGTA ACGCTAATT CAAAGCTATC ACAAATTTC CTTTTAATGT
3101 AGGCGGCTTG TTGACAACA AAGGCAATTC AAATATTTC ATTGCCAAAG
3151 GAGGGGCTCG CTTTAAAGAC ATTGATAATT CCAAGAAATT AAGCATCACC
3201 ACCAACTCCA GCTCCACTTA CCGCACTATT ATAAGCGGCA ATATAACCAA
3251 TAAAAACGGT GATTAAATA TTACGAACGA AGGTAGTAT ACTGAAATGC

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25/73

FIG. 6E.

3301 AAATTGGCGG CGATGTCTCG CAAAAGAAG GTAATCTCAC GATTCTTCT
3351 GACAAAATCA ATATTACCA ACAGATAACA ATCAAGGCAG GTGTTGATGG
3401 GGAGAAATCC GATTCAGACG CGACAAACAA TGCCAATCTA ACCATTAAAA
3451 CCAAAGAATT GAAATTAACG CAAGACCTAA ATATTTCAGG TTTCAATAAA
3501 GCAGAGATTA CAGCTAAAGA TGGTAGTGAT TTAACATAATG GTAACACCAA
3551 TAGTGCTGAT GGTACTAATG CCAAAAAAGT AACCTTTAAC CAGGTTAAAG
3601 ATTCAAAAAT CTCTGCTGAC GGTCACAAGG TGACACTACA CAGCAAAGTG
3651 GAAACATCCG GTAGTAATAA CAACACTGAA GATAGCAGTG ACAATAATGC
3701 CGGCTTAAC TCGATGCAA AAAATGTAAC AGTAAACAAC AATATTACTT
3751 CTCACAAAGC AGTCAGCATC TCTGCGACAA GTGGAGAAAT TACCCTAAA
3801 ACAGGTACAA CCATTACGC AACCACTGGT AACGTGGAGA TAACCGCTCA
3851 AACAGGTAGT ATCCTAGGTG GAATTGAGTC CAGCTCTGGC TCTGTAACAC
3901 TTAAGTCAAC CGAGGGCGCT CTGCTGTAA GCAATATTTC GGGCAACACC
3951 GTTACTGTTA CTGCAAAATAG CGGTGCATTA ACCACTTTGG CAGGCTCTAC
4001 AATTAAAGGA ACCGAGAGTG TAACCACTTC AAGTCAATCA GCGATATCG
4051 GCGGTACCAT TTCTGGTGGC ACAGTAGAGG TTAAAGCAAC CGAAAGTTTA

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FIG. 6F.

4101 ACCACTCAAT CCAATTCAAA AATTAAAGCA ACAACAGGCG AGGCTAACGT
4151 AACAAAGTGCA ACAGGTACAA TTGGTGGTAC GATTTCGGT AATACGGTAA
4201 ATGTTACGGC AAACGCTGGC GATTTAACAG TTGGGAATGG CGCAGAAAT
4251 AATGCGACAG AAGGAGCTGC AACCTTAACT ACATCATCGG GCAAATTAAAC
4301 TACCGAAGCT AGTTCACACA TTACTTCAGC CAAGGTCAG GTAAATCTTT
4351 CAGCTCAGGA TGGTAGCGTT GCAGGAAGTA TTAATGCCGC CAATGTGACA
4401 CTAATAACTA CAGGCACTTT AACTACCGTG AAGGGTTCAA ACATTAATGC
4451 AACCAGCGGT ACCTTGGTTA TTAACGCCAA AGACGCTGAG CTAAATGGCG
4501 CAGCATTTGG TAACCACACA GTGTAATG CAACCAACGC AAATGGCTCC
4551 GGCAGCGTAA TCGCGACAAC CTCAGCAGA GTGAACATCA CTGGGGATTT
4601 AATCACAAATA AATGGATTAA ATATCATTTT AAAAAACGGT ATAAACACCG
4651 TACTGTTAAA AGGCGTTAAA ATTGATGTGA AATACATTCA ACCGGGTATA
4701 GCAAGCGTAG ATGAAGTAAT TGAAGCGAAA CGCATCCTTG AGAAGGTAAA
4751 AGATTATCT GATGAAGAAA GAGAAGCGTT AGCTAAACTT GCGTAAAGTG
4801 CTGTACGTTT TATTGAGCCA AATAATACAA TTACAGTCGA TACACAAAAT
4851 GAATTGCAA CCAGACCATT AAGTCGAATA GTGATTTCTG AAGGCAGGGC
4901 GTGTTTCTCA AACAGTGATG GCGGACGGT GTGCGTTAAT ATCGTGATA

26/73

RECTIFIED SHEET (RULE 91)

27/23

FIG. 6G.

4951 ACGGGCGGTA GCGGTCAGTA ATTGACAAGG TAGATTTTCAT CCTGCAATGA
5001 AGTCATTTTA TTTTCGTATT ATTTACTGTG TGGGTTAAAG TTCAGTACGG
5051 GCTTTACCCA TCTTGTA AAA AATTACGGAG AATACAAATA AGTATTTTTA
5101 ACAGGTATAT ATTATGAAAA ATATAAAAG CAGATTAAAA CTCAGTGCAA
5151 TATCAGTATT GCTTGGCCTG GCTTCTTCAT CATTGTATGC AGAAGAAGCG
5201 TTTT TAGTAA AAGGCTTTCA GTTATCTGGT GCACTTGAAA CTTTAAGTGA
5251 AGACGCCCAA CTGCTCTGTAG CAAAATCTTT ATCTAAATAC CAAGGCTCGC
5301 AAACCTTTAAC AAACCTAAAA ACAGCACAGC TTGAAATTACA GGCTGTGCTA
5351 GATAAGATTG AGCCAAATAA GTTTGATGTG ATATTGCCAC AACAAACCAT
5401 TACGGATGGC AATATTATGT TTGAGCTAGT CTCGAAATCA GCCGCAGAAA
5451 GCCAAGTTTT TTATAAGCG AGCCAGGGTT ATAGTGAAGA AAATATCGCT
5501 CGTAGCCTGC CATCTTTGAA ACAAGGAAA GTGTATGAAG ATGGTCGTCA
5551 GTGGTTCGAT TTGCGTGAAT TCAATATGGC AAAAGAAAAAT CCAC'TTAAAG
5601 TCACTCGCGT GCATTACGAG TTAAACCCTA AAAACAAAC CTCGTATTG
5651 GTAGTTGCAG GTTTTTCGCC TTTTGGCAA ACGCGTAGCT TTGTTTCCCTA
5701 TGATAATTTC GCGCAAGGG AGTTTAACTA TCAACGTGTA AGCTAGGTT

RECTIFIED SHEET (RULE 91)

28/73

FIG. 6H.

5751 TTGTAAATGC CAATTGACC GGACATGATG ATGTATTAAA TCTAAACGCA
 5801 TTGACCAATG TAAAGCACC ATCAAAATCT TATGCCGGTAG GCATAGGATA
 5851 TACTTATCCG TTTTATGATA AACACCAATC CTTAAGTCTT TATACCAGCA
 5901 TGAGTTATGC TGATTCCTAAT GATATCGACG GCTTACCAAG TGGATTAAAT
 5951 CGTAAATTAT CAAAAGGTCA ATCTATCTCT GCGAATCTGA AATGGAGTTA
 6001 TTATCTCCCG ACATTTAACC TTGGAATGGA AGACCAGTTT AAAATTAAAT
 6051 TAGGCTACAA CTACCGCCAT ATTAATCAAA CATCCGAGTT AAACACCCCTG
 6101 GGTGCAACGA AGAAAAAATT TGCAGTATCA GGCGTAAGTG CAGGCATTGA
 6151 TGGACATATC CAATTTACCC CTAAACAAT CTTTAATATT GATTTAACTC
 6201 ATCATTTATTA CGCGAGTAAA TTACCAGGCT CTTTGTGAAT GGAGCGCATT
 6251 GCGAAACAT TTAATCGCAG CTATCACATT AGCACAGCCA GTTTAGGGTT
 6301 GAGTCAAGAG TTTGCTCAAG GTTGGCATT TAGCAGTCAA TTATCGGGTC
 6351 AGTTTACTCT ACAAGATATA AGTAGCATAG ATTTATTCTC TGTAACAGGT
 6401 ACTTATGGCG TCAGAGGCTT TAAATACGGC GGTGCAAGTG GTGAGCGCGG
 6451 TCTTGTATGG CGTAATGAAT TAAGTATGCC AAAATACACC CGCTTTCAAA
 6501 TCAGCCCTTA TCGGTTTAT GATGCAGGTC AGTTCCGTTA TAATAGCGAA
 6551 AATGCTAAAA CTTACGGCGA AGATATGCAC ACGGTATCCT CTGCGGGTTT

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29/23

FIG. 61.

6601 AGGCATTAAA ACCTCTCCTA CACAAACTT AAGCTTAGAT GCTTTTGTG
6651 CTCGTCGCTT TGCAAATGCC AATAGTGACA ATTTGAATGG CAACAAAAA
6701 CGCACAAAGCT CACCTACAAC CTTCCTGGGT AGATTAAACAT TCAGTTTCTA
6751 ACCCTGAAAT TTAATCAACT GGTAAGCGTT CCGCC'TACCA GTTTATAACT
6801 ATATGCTTTA CCCGCCAATT TACAGTCTAT ACGCAACCCT GTTTTCATCC
6851 TTATATATCA AACAACTAA GCAAAACCAAG CAAACCAAGC AAACCAAGCA
6901 AACCAAGCAA ACCAAGCAA CCAAGCAAAC CAGCAAACC AAGCAAACCA
6951 AGCAAACCAA GCAAACCAAG CAAACCAAGC AAACCAAGCA ATGCTAAAAA
7001 ACAATTTATA TGATAAACTA AAACATATCT CATACCATGG CAATACAAGG
7051 GATTTAATAA TATGACAAAA GAAATTTAC AAAGTGTTC ACAAATACG
7101 ACCGCTTCAC TTGTAGAATC AAACAACGAC CAAACTTCCC TGCAAATACT
7151 TAAACAACCA CCCAAACCCA ACCTATTACG CCTGGAACAA CATGTCGCCA
7201 AAAAAGATTA TGAGCTTGCT TGCCGCGAAT TAATGGCGAT TTTGGAAAAA
7251 ATGGACGCTA ATTTTGAGG CGTTCACGAT ATTGAATTG ACGCACCTGC
7301 TCAGCTGGCA TATCTACCCG AAAAATACT AATTCATTT GCCACTCGTC
7351 TCGCTAATGC AATTACAACA CTCTTTTCCG ACCCCGAATT GGCAATTTC

RECTIFIED SHEET (RULE 91)

FIG. 6J.

30/23

7401	GAAGAAGGG	CATTAAGAT	GATTAGCCTG	CAACGCTGGT	TGACGCTGAT
7451	TTTTGCTCT	TCCCCCTACG	TTAACGCAGA	CCATATTCTC	AATAAATATA
7501	ATATCAACCC	AGATTCCGAA	GGTGGCTTTC	ATTTAGCAAC	AGACAACTCT
7551	TCTATTGCTA	AATTCTGTAT	TTTTTACTTA	CCCGAATCCA	ATGTCAATAT
7601	GAGTTTAGAT	GCGTTATGGG	CAGGGAATCA	ACAACTTTGT	GCTTCATTGT
7651	GTTTGGCGTT	GCAGTCTTCA	CGTTTATTG	GTA CTGCATC	TGCGTTTCAT
7701	AAAAGAGCGG	TGGTTT TACA	GTGGTTTCCT	AAAAAACTCG	CCGAAATTGC
7751	TAATTTAGAT	GAATTGCCCTG	CAAATATCCT	TCATGATGTA	TATATGCACT
7801	GCAGTTATGA	TTTAGCAAAA	AACAAGCACG	ATGTTAAGCG	TCCATTAAAC
7851	GAAC TTGTCC	GCAAGCATAT	CCTCACGCAA	GGATGGCAAG	ACCGCTACCT
7901	TTACACCTTA	GGTAAAAGG	ACGGCAAACC	TGTGATGATG	GTACTGCTTG
7951	AACATTTTAA	TTCGGGACAT	TCGATTTATC	GCACGCATTC	AACTTCAATG
8001	ATTGCTGCTC	GAGAAAAATT	CTATTTAGTC	GGCTTAGGCC	ATGAGGGCGT
8051	TGATAACATA	GGTCGAGAAG	TGTTTGACGA	GTTCTTTGAA	ATCAGTAGCA
8101	ATAATATAAT	GGAGAGACTG	TTTTTTTATCC	GTAAACAGTG	CGAAACTTTC
8151	CAACCCGCAG	TGTTCTATAT	GCCAAGCATT	GGCATGGATA	TTACCACGAT

RECTIFIED SHEET (RULE 91)

31/23

FIG. 6K.

8201 TTTTGTGAGC AACACTCGGC TTGCCCCCTAT TCAAGCTGTA GCCTTGGGTC
 8251 ATCCTGCCAC TACGCATTCT GAATTATG ATATGTCAT CGTAGAAGAT
 8301 GATTATGTGG GCAGTGAAGA TTGTTTAGC GAAACCCCTT TACGCTTACC
 8351 CAAAGATGCC CTACCTTATG TACCATCTGC ACTCGCCCCA CAAAAAGTGG
 8401 ATTATGTACT CAGGGAAAAC CCTGAAGTAG TCAATATCGG TATTGCCGCT
 8451 ACCACAATGA AATTAAACCC TGAATTTTGG CTAACATTGC AAGAAATCAG
 8501 AGATAAAGCT AAAGTCAAAA TACATTTTCA TTTCGCACTT GGACAATCAA
 8551 CAGGCTTGAC ACACCCCTTAT GTCAAATGGT TTATCGAAAG CTATTTAGGT
 8601 GACGATGCCA CTGCACATCC CCACGCACCT TATCACCATT ATCTGGCAAT
 8651 ATTGCGTGAT TCGGATATGC TACTAAATCC GTTTCCTTTC GGTAAACTA
 8701 ACGGCATAAT TGATATGGTT ACATTAGGTT TAGTTGGTGT ATGCAAAACG
 8751 GGGGATGAAG TACATGAACA TATTGATGAA GGCTGTGTTA AACGCTTAGG
 8801 ACTACCAGAA TGGCTGATAG CCGACACACG AGAAACATAT ATTGAATGTG
 8851 CTTTGCGTCT AGCAGAAAAC CATCAAGAAC GCCTTGAACT CCGTCGTTAC
 8901 ATCATAGAAA ACAACGGCTT ACAAAAGCTT TTACAGGCG ACCCTCGTCC
 8951 ATTGGGCAAA ATACTGCTTA AGAAAACAAA TGAATGGAAG CGGAAGCACT
 9001 TGAGTAAAAA ATAACGGTTT TTAAAGTAA AAGTGGGTT AATTTTCAAA

RECTIFIED SHEET (RULE 91)

32/73

FIG. 6L.

9051 GCGTTTAA AACCTCTCAA AAATCAACCG CACTTTTATC TTTATAACGC
9101 TCCCGCGCGC TGACAGTTA TCTCTTCTT AAAATACCCA TAAATTTGTG
9151 GCAATAGTTG GGTAATCAAA TTCAATTGTT GATACGGCAA ACTAAAGACG
9201 GCGCGTTCTT CGGCAGTCAT C

RECTIFIED SHEET (RULE 91)

33/73

FIG. 7A.

1 CGCCACTTCA ATTTTGGATT GTTGAAATTC AACTAACCAG AAAGTGCGGT
 51 TAAAATCTGT GGAGAAAATA GGTGTAGTG AAGAACGAGG TAATTGTTCA
 101 AAAGGATAAA GCTCTCTTAA TTGGGCATG GTTGGCGTTT CTTTTCGGT
 151 TAATAGTAAA TTATATTCTG GACGACTATG CAATCCACCA ACAACTTTAC
 201 CGTTGGTTT AAGCGTTAAT GTAAGTTCTT GCTCTTCTTG GCGAATACGT
 251 AATCCCATTT TTTGTTTAGC AAGAAAATGA TCGGGATAAT CATAATAGGT
 301 GTTGCCCAA AATAAATTTT GATGTTCTAA AATCATAAAT TTGCAAGAT
 351 ATTGTGGCAA TTCAATACCT ATTTGTGGG AAATCGCCAA TTTTAATTCA
 401 ATTTCTTGTA GCATAATATT TCCCACCTCA ATCAACTGGT TAAATATACA
 451 AGATAATAAA AATAAATCAA GATTTTGTG ATGACAAACA ACAATTACAA
 501 CACCTTTTTT GCAGTCTATA TGCAAATATT TTAATAAAT AGTATAAATC
 551 CGCCATATAA AATGGTATAA TCTTTCATCT TTCATCTTTC ATCTTTCATC
 601 TTTCATCTTT CATCTTTCAT CTTTCATCTT TCATCTTTC TCTTTCATCT
 651 TTTCATCTTTC ATCTTTCATC TTTTCATCTT CACATGAAAT GATGAACCGA
 701 GGGAAGGGAG GGAGGGGCAA GAATGAAGAG GGAGCTGAAC GAACGCAAT
 751 GATAAAGTAA TTTAATTGTT CAACTAACCT TAGGAGAAA TATGAACAAG

RECTIFIED SHEET (RULE 91)

34/23

FIG. 7B.

801 ATATATCGTC TCAAATTCAG CAAACGCCTG AATGCTTTGG TTGCTGTGTC
 851 TGAATGGCA CGGGTTGTG ACCATTCCAC AGAAAAAGGC AGCGAAAAAC
 901 CTGCTCGCAT GAAAGTGCGT CACTTAGCGT TAAAGCCACT TTCCGCTATG
 951 TTAATACTT TAGGTGTAA ATCTATTCCA CAATCTGTTT TAGCAAGCGG
 1001 CAATTTAACA TCGACCAAAA TGAAATGGTG CAGTTTTTAC AAGAAAAACAA
 1051 GTAATAAAC CATTATCCGC AACAGTGTG ACGCTATCAT TAATTGGAAA
 1101 CAATTTAACA TCGACCAAAA TGAAATGGTG CAGTTTTTAC AAGAAAAACAA
 1151 CAACTCCGCC GTATTCAACC GTGTTACATC TAACCAAAATC TCCCAATTAA
 1201 AAGGGATTTT AGATTCTAAC GGACAAGTCT TTTTAATCAA CCCAAATGGT
 1251 ATCACAAATAG GTAAGACGC AATTATTAAC ACTAATGGCT TTACGGCTTC
 1301 TACGCTAGAC ATTTCTAACG AAAACATCAA GGCGCGTAAT TTCACCTTCG
 1351 AGCAAACCAA AGATAAAGCG CTCGCTGAAA TTGTGAATCA CGGTTTAATT
 1401 ACTGTCGGTA AAGACGGCAG TGTAAATCTT ATTGGTGGCA AAGTGAAAAA
 1451 CGAGGTGTG ATTAGCGTAA ATGGTGGCAG CATTCTTTA CTCGCAGGGC
 1501 AAAAAATCAC CATCAGCGAT ATAATAAACC CAACCATTAC TTACAGCATT
 1551 GCCGCGCCTG AAAATGAAGC GGTCAATCTG GCGATATTT TTGCCAAAGG

RECTIFIED SHEET (RULE 91)

35/73

FIG. 7C.

1601 CGGTAACATT AATGTCCGTG CTGCCACTAT TCGAAACCAA GGTAACCTTT
 1651 CTGCTGATTTC TGTAAGCAAA GATAAAGCG GCAATATTGT TCTTTCCGCC
 1701 AAAGAGGGTG AAGCGGAAAT TGGCCGTGTA ATTTCGGCTC AAAATCAGCA
 1751 AGCTAAAGGC GGCAAGCTGA TGATTACAGG CGATAAAGTC ACATTAATAA
 1801 CAGGTGCAGT TATCGACCTT TCAGGTAAG AAGGGGAGA AACTTACCTT
 1851 GGCGGTGACG AGCGCGGCGA AGGTAAAAC GGCAATCAAT TAGCAAAAGAA
 1901 AACCTCTTTA GAAAAGGCT CAACCATCAA TGATATCAGGC AAAGAAAAG
 1951 GCGGACGCGC TATTCTGTGG GCGGATATTG CGTTAAATTGA CCGCAATATT
 2001 AACGCTCAAG GTAGTGGTGA TATCGCTAAA ACCGGTGGTT TTGTGGAGAC
 2051 ATCGGGGCAT TATTATATCCA TTGACAGCAA TGCAATTGTT AAAACAAAAG
 2101 AGTGGTTGCT AGACCTTGAT GATGTAACAA TTGAAGCCGA AGACCCCTTT
 2151 CGCAATAATA CCGGTATATA TGATGAATTC CCAACAGGCA CCGGTGAAGC
 2201 AAGCGACCCCT AAAAATAAATA GCGAACTCAA AACAAACGCTA ACCAATACAA
 2251 CTATTTCAA TTTATCTGAAA AACGCCCTGA CAATGAATAT AACGGCATCA
 2301 AGAAAACCTTA CCGTTAATAG CTCAATCAAC ATCGGAAGCA ACTCCCACTT
 2351 AATTCTCCAT AGTAAAGGTC AGCGTGGCGG AGGCGTTTCA ATTGATGGAG
 2401 ATATTACTTC TAAAGGCGGA AATTAAACCA TTTATTCTGG CGGATGGGTT

RECTIFIED SHEET (RULE 91)

36/73

FIG. 7D.

2451 GATGTTGATA AAAATATTAC GCTTGATCAG GTTTTTTTAA ATATTACCGC
 2501 CGCTTCCGTA GCTTTTGAAG GTGGAAATAA CAAAGCACGC GACGCGGCAA
 2551 ATGCTAAAAT TGTGCGCCAG GGCACGTGTA CCATTACAGG AGAGGGAAAA
 2601 GATTTCAGGG CTAACAACGT ATCTTTAAAC GGAACGGGTA AAGGTCTGAA
 2651 TATCATTTCA TCAGTGAATA ATTTAACCCA CAATCTTAGT GGCACAATTA
 2701 ACATATCTGG GAATATAACA ATTAACCAAA CTACGGAGAA GAACACCTCG
 2751 TATTGGCAA CCAGCCATGA TTCGCACTGG AACGTCAGTG CTCTTAATCT
 2801 AGAGACAGGC GCAAATTTTA CCTTTATTAA ATACATTTCA AGCAATAGCA
 2851 AAGGCTTAAC AACACAGTAT AGAAGCTCTG CAGGGGTGAA TTTTAACGGC
 2901 GTAAATGGCA ACATGTCATT CAATCTCAA GAAGGAGCGA AAGTTAATT
 2951 CAAATTAAAA CCAAACGAGA ACATGAACAC AAGCAAACCT TTACCAATTC
 3001 GGTTTTTCAG CAATATCACA GCCACTGGTG GGGGCTCTGT TTTTTCATGAT
 3051 ATATATGCCA ACCATTCTGG CAGAGGGGCT GAGTTAAAA TGAGTGAAAT
 3101 TAATATCTCT AACGGCGCTA ATTTTACCTT AAATTCCCAT GTTCGCGGCG
 3151 ATGACGCTTT TAAAATCAAC AAAGACTTAA CCATAAATGC AACCAATTCA
 3201 AATTTCAGCC TCAGACAGAC GAAAGATGAT TTTTATGACG GTACGCACG

RECTIFIED SHEET (RULE 91)

37/73

FIG. 7E.

3251 CAATGCCATC AATCAACCT ACAACATATC CATCTGGGC GGTAATGTCA
 3301 CCCTTGGTGG ACAAACCTCA AGCAGCAGCA TTACGGGGAA TATTACTATC
 3351 GAGAAAGCAG CAAATGTTAC GCTAGAAGCC AATAACGCCC CTAATCAGCA
 3401 AAACATAAGG GATAGAGTTA TAAAACTTGG CAGCTTGCTC GTTAATGGGA
 3451 GTTTAAGTTT AACTGGCGAA AATGCAGATA TTAAAGGCAA TCTCACTATT
 3501 TCAGAAAGCG CCACTTTTAA AGGAAAGACT AGAGATACCC TAAATATCAC
 3551 CGGCAATTTT ACCAATAATG GCACTGCCGA AATTAAATATA ACACAAGGAG
 3601 TGGTAAAACT TGGCAATGTT ACCAATGATG GTGATTTAAA CATTACCACT
 3651 CACGCTAAAC GCAACCAAAG AAGCATCATC GGCGGAGATA TAATCAACAA
 3701 AAAAGGAAGC TTAAATATTA CAGACAGTAA TAATGATGCT GAAATCCAAA
 3751 TTGGCGGCAA TATCTCGCAA AAAGAAGGCA ACCTCACGAT TTCTTCCGAT
 3801 AAAATTAAATA TCACCAAACA GATAACAATC AAAAAGGGTA TTGATGGAGA
 3851 GGACTCTAGT TCAGATGCCA CAAGTAATGC CAACCTAACT ATTAAAACCA
 3901 AAGAAATTGAA ATTGACAGAA GACCTAAGTA TTTCAGGTTT CAATAAAGCA
 3951 GAGATTACAG CCAAAGATGG TAGAGATTTA ACTATTGGCA ACAGTAATGA
 4001 CGGTAACAGC GTGCGGAAG CCAAAACAGT AACTTTTAAC AATGTTAAAG

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FIG. 7F.

4051 ATTCAAAAAT CTCTGCTGAC GGTCACAATG TGACACTAAA TAGCAAAGTG
 4101 AAAACATCTA GCAGCAATGG CGGACGTGAA AGCAATAGCG ACAACGATAC
 4151 CGGCTTAACT ATTACTGCAA AAAATGTAGA AGTAAACAAA GATATTACTT
 4201 CTC'TCAAAAC AGTAAATATC ACCGCGTCGG AAAAGGTTAC CACCACAGCA
 4251 GGCTCGACCA TTAACGCAAC AAATGGCAA GCAAGTATTA CAACCAAAAC
 4301 AGGTGATATC AGCGGTACGA TTTCCGGTAA CACGGTAAGT GTTAGCGCGA
 4351 CTGGTGATTT AACCACTAAA TCCGGCTCAA AAATGAAGC GAAATCGGGT
 4401 GAGGCTAATG TAACAAGTGC AACAGGTACA ATTGGCGGTA CAATTTCGGG
 4451 TAATACGGTA AATGTTACGG CAAACGCTGG CGATTTAACA GTTGGGAATG
 4501 GGCAGAAAT TAATGCCACA GAAGGAGCTG CAACCTTAAC CGCAACAGGG
 4551 AATACCTTGA CTA CTGAAGC CGGTTCTAGC ATCACTTCAA CTAAGGGTCA
 4601 GGTAGACCTC TTGGCTCAGA ATGGTAGCAT CGCAGGAAGC ATTAATGCTG
 4651 CTAATGTGAC ATTAAATACT ACAGGCACCT TAACCCACCGT GGCAGGCTCG
 4701 GATATTTAAAG CAACCAGCGG CACCTTGGTT ATTAACGCAA AAGATGCTAA
 4751 GCTAAATGGT GATGCATCAG GTGATAGTAC AGAAGTGAAT GCAGTCAACG
 4801 ACTGGGGATT TGGTAGTGTG ACTGCGGCAA CCTCAAGCAG TGTGAATATC
 4851 ACTGGGGATT TAAACACAGT AAATGGGTTA AATATCATTT CGAAAGATGG

38/73

RECTIFIED SHEET (RULE 91)

39/73

FIG. 7G.

4901 TAGAAACACT GTCCGCTTAA GAGCAAGGA AATTGAGGTG AAATATATCC
 4951 AGCCAGGTGT AGCAAGTGTA GAAGAAGTAA TTGAAGCGAA ACGCGTCCCTT
 5001 GAAAAGTAA AAGATTATC TGATGAGAA AGAGAAACAT TAGCTAAACT
 5051 TGGTGTAAAGT GCTGTACGTT TTGTTGAGCC AAATAATACA ATTACAGTCA
 5101 ATACACAAAA TGAATTTACA ACCAGACCGT CAAGTCAAGT GATAATTCT
 5151 GAAGGTAAGG CGTGTTTCTC AAGTGGTAAT GGCGCACGAG TATGTACCAA
 5201 TGTTGCTGAC GATGGACAGC CGTAGTCAGT AATTGACAAG GTAGATTCA
 5251 TCCTGCAATG AAGTCA'TTTT ATTTTCGTAT TATTACTGT GTGGGTAA
 5301 GTTCAGTACG GGCTTTACCC ATCTTGTA
 5351 AAGTATTTTT AACAGGTTAT TATTATGAAA AATATAAAA GCAGATTAAA
 5401 ACTCAGTGCA ATATCAGTAT TGCTTGGCCT GCCTTCTTCA TCATTGTATG
 5451 CAGAAGAAGC GTTTT'TAGTA AAAGGCTTTC AGTTATCTGG TGCACCTGAA
 5501 ACTTTAAGTG AAGACGCCCA ACTGTCTGTA GCAAAATCTT TATCTAAATA
 5551 CCAAGGCTCG CAAACTTTAA CAAACCTAAA AACAGCACAG CT'TGAATTAC
 5601 AGGCTGTGCT AGATAAGATT GAGCCAAATA AATTGATGT GATATTGCCG
 5651 CAACAAACCA TTACGGATGG CAATATCATG TTGAGCTAG TCTCGAAATC

RECTIFIED SHEET (RULE 91)

FIG. 7H.

5701 AGCCGCAGAA AGCCAAGTTT TTTATAAGC GAGCCAGGGT TATAGTGAAG
 5751 AAAATATCGC TCGTAGCCTG CCATCTTTGA AACAAAGGAAA AGTGTATGAA
 5801 GATGGTCGTC AGTGGTTTCGA TTTGCGGTGAA TTTPAATATGG CAAAAGAAAA
 5851 CCCGCTTAAG GTTACCCGTG TACATTACGA ACTAAACCCCT AAAAACAAAA
 5901 CCTCTAATTT GATAATTGCG GGCTTCTCGC CTTT'TGGTAA AACGCGTAGC
 5951 TT'TATTTCTT ATGATTAATTT CGGCCCGAGA GAGTTTAACT ACCAACGTC
 6001 AAGCTTGGGT TTTGTTAATG CCAATTTAAC TGGTCATGAT GATGTGTTPAA
 6151 TTATACCAGT ATGAGTTATG CTGATTCTAA TGATATCGAC GGCTTACCAA
 6201 GTGCGATTAA TCGTAAATTA TCAAAAGGTC AATCTATCTC TGGGAATCTG
 6251 AAATGGAGTT ATTATCTCCC AACATTTAAC CT'TGGCATGG AAGACCAATP
 6301 TAAAATTAAT TTAGGCTPACA ACTACCGCCA TATTTAATCAA ACC'TCCCGT
 6351 TAAATCGCTT GGGTGAAACG AAGAAAAAAT TTGCAGTATC AGCGGTAAGT
 6401 GCAGGCATTG ATGGACATAT CCAATTTACC CCTAAAACAA TCCTTAATAAT
 6451 TGATTTAACT CATCATTAAT ACGCGAGTAA ATTACCAGGC TCTTTTGGAA
 6501 TGGAGCGCAT TGGCGAAACA TT'TAATCGCA GCTATCACAT TAGCACAGCC
 6551 AGTTTAGGGT TGAGTCAAGA GTTTGCTCAA GGTGGCATT TTAGCAGTCA
 6601 ATTATCAGGT CAATTTACTC TACAAGATAT TAGCAGTATA GATTATTCT

40/23

RECTIFIED SHEET (RULE 91)

41/73

FIG. 7I.

6651	CTGTAACAGG	TACTTATGGC	GTCAGAGGCT	TTAATACGG	CGTGCAAGT
6701	GGTGAGCGCG	GTCCTGTATG	GGTAATGAA	TTAAGTATGC	CAAAATACAC
6751	CCGCTTCCAA	ATCAGCCCTT	ATGCGTTTTA	TGATGCAGGT	CAGTTCCGTT
6801	ATAATAGCGA	AAATGCTAAA	ACTTACGGCG	AAGATATGCA	CACGGTATCC
6851	TCTGCGGGTT	TAGGCATTAA	AACCTCTCCT	ACACAAAAC	TAAAGCCTAGA
6901	TGCTTTTGTT	GCTCGTCGCT	TTGCCAAATGC	CAATAGTGAC	AATTGAATG
6951	GCAACAAAAA	ACGCACAAGC	TCACCTACAA	CCTTCTGGGG	GAGATTAAAC
7001	TTCAGTTTCT	AACCCTGAAA	TTTAATCAAC	TGGTAAGCGT	TCCGCCCTACC
7051	AGTTTATAAC	TATATGCTTT	ACCCGCCCAAT	TTACAGTCTA	TAGGCAACCC
7101	TGTTTTTACC	CTTATATATC	AAATAAACAA	GCTAAGCTGA	GCTAAGCAAA
7151	CCAAGCAAAC	TCAAGCAAGC	CAAGTAATAC	TAAAAAAACA	ATTATATGA
7201	TAAACTAAAG	TATACTCCAT	GCCATGGCGA	TACAAGGGAT	TTAATAATAT
7251	GACAAAAGAA	AATTTGCAAA	ACGCTCCTCA	AGATGCGACC	GCTTTACTTG
7301	CGGAATTAAG	CAACAATCAA	ACTCCCCCTGC	GAATATTAA	ACAACCACGC
7351	AAGCCCAGCC	TATTACGCTT	GGAACAACAT	ATCGCAAAA	AAGATTATGA
7401	GTTTGCTTGT	CGTGAATTAA	TGGTGATTCT	GGAAAAAATG	GACGCTAAT

RECTIFIED SHEET (RULE 91)

42/82

FIG. 7J.

7451 TTGGAGGCGT TCACGATATT GAATTGACG CACCCGCTCA GCTGGCATAT
7501 CTACCCGAAA AATACTAAT TTATTTTGCC ACTCGTCTCG CTAATGCAAT
7551 TACAACACTC TTTTCCGACC CCGAATTGGC AATTCTGAA GAAGGGCGGT
7601 TAAAGATGAT TAGCCTGCAA CGCTGGTTGA CGCTGATTTT TGCCCTCTCC
7651 CCCTACGTTA ACGCAGACCA TATTCTCAAT AAATATAATA TCAACCCAGA
7701 TTCCGAAGGT GGCTTTCATT TAGCAACAGA CAACTCTTCT ATTGCTAAAT
7751 TCTGTATTTT TTA CTTACCC GAATCCAATG TCAATATGAG TTTAGATGCG
7801 TTATGGGCAG GGAATCAACA ACTTTGTGCT TCATTGTGTT TTGCGTTGCA
7851 GTCTTCACGT TTTATTTGTA CCGCATCTGC GTTTCATAAA AGAGCGGTGG
7901 TTTTACAGTG GTTTCCTAAA AAACTCGCCG AAATTGCTAA TTTAGATGAA
7951 TTGCCTGCAA ATATCCTTCA TGATGTATAT ATGCACTGCA GTTATGATT
8001 AGCAAAAAAC AAGCACGATG TTAAGCGTCC ATTAAACGAA CTTGTCCGCA
8051 AGCATATCCT CACGCAAGGA TGGCAAGACC GCTACCTTTA CACCTTAGGT
8101 AAAAAGGACG GCAAACCTGT GATGATGGTA CTGCTTGAAC ATTTTAATTC
8151 GGGACATTTCG ATTTATCGTA CACATTCAAC TTCAATGATT GCTGCTCGAG
8201 AAAAATTCTA TTTAGTCGGC TTAGGCCATG AGGGCGTTGA TAAAAATAGGT

RECTIFIED SHEET (RULE 91)

43/73

FIG. 7K.

8251 CGAGAAAGTGT TTGACGAGTT CTTTGAATC AGTAGCAATA ATATAATGGA
 8301 GAGACTGTTT TTTATCCGTA AACAGTCCGA AACTTTCCAA CCCGCAGTGT
 8351 TCATATATGCC AAGCAATTGCC ATGGATATTA CCACGATTTT TGTGAGCAAC
 8401 ACTCGGCTTG CCCCTATTCA AGCTGTAGCC CTGGGTCATC CTGCCACTAC
 8451 GCATTCTGAA TTTATTTGATT ATGTCATCGT AGAAGATGAT TATGTGGCA
 8501 GTGAAGATTG TTTGAGCGAA ACCCTTTTAC GCTTACCCAA AGATGCCCTA
 8551 CCTTATGTAC CTTCCTGCACT CGCCCCACAA AAAGTGATT ATGTACTCAG
 8601 GGAAAAACCCCT GAAGTAGTCA ATATCGGTAT TGCCCGCTACC ACAAATGAAAT
 8651 TAAACCCCTGA ATTTTTGGCTA ACATTGCAAG AAATCAGAGA TAAAGCTAAA
 8701 GTCAAAATAC ATTTTTCATTT CGCACTTGGA CAATCAACAG GCTTGACACA
 8751 CCTTATGTC AAATGGTTTA TCGAAAGCTA TTTAGGTGAC GATGCCACTG
 8801 CACATCCCCA CGCACCTTAT CACGATTATC TGGCAATATT GCGTGAATTG
 8851 GATATGCTAC TAAATCCGTT TCCTTTCGGT AATACTAACG GCATAATTGA
 8901 TATGGTTACA TTAGGTTTAG TTGGTGTATG CAAAACGGGG GATGAAGTAC
 8951 ATGAACATAT TGATGAAGGT CTGTTTAAAC GCTTAGGACT ACCAGAAATGG
 9001 CTGATAGCCG ACACACGAGA AACATATATT GAATGTGCTT TCGGCTCTAGC
 9051 AGAAAACCAT CAAGAACGCC TTGAACTCCG TCGTTACATC ATAGAAAACA

RECTIFIED SHEET (RULE 91)

44/73

FIG. 7L.

9101 ACGGCTTACA AAAGCTTTT ACAGGCGACC CTCGTCCATT GGGCAAAATA
9151 CTGCTTAAGA AACAAATGA ATGGAAGCGG AAGCACTGA GTAAAAAATA
9201 ACGGTTTTT AAAGTAAAAG TCGGGTTAAT TTTCAAAGCG TTTTAAAAAC
9251 CTCTCAAAA TCAACCGCAC TTTTATCTTT ATAACGATCC CGCACGCTGA
9301 CAGTTTATCA GCCTCCCGCC ATAAACTCC GCCTTTCATG GCGGAGATTT
9351 TAGCCAAAC TGGCAGAAAT TAAAGGCTAA AATCACC AAA TTGCACCACA
9401 AAATCACCAA TACCCACAAA AAA

RECTIFIED SHEET (RULE 91)

45/73

F. 8

HMW3 nucleotide sequence

F. 8

REFORMAT of: Temp3.Gcg check: -1 from: 1 to: 4794 October 5, 1995 17:43

(No documentation)

Hmw3.Gcg Length: 4794 October 5, 1995 18:29 Type: M Check: 484 ..

1 ATGAACAAGA TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT TGCTGTGTCT GAATTGACAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA
101 GTGAAAAACC TGTCGTACG AAAGTACGCC ACTTGGCGTT AAAGCCACTT TCGGTATAT TGCTATCTTT GGGCATGGCA TCCATTCGCC AATCTGTTT
201 AGCGAGCGGT TTACAGGGAA TGAGCGTCT ACACGGTACA GCAACCATGC AAGTAGACGG CAATAAAACC ACTATCCGTA ATAGCGTCAA TGCTATCATC
301 AATTGGAAAC AATTTAATCAT TGACCAAAAT GAAATGGTGC AGTTTTTACA AGAAAGCAGC AACTCTGCCG TTTTCAACCG TGTACATCT GACCAATCT
401 CCAATTAAA AGGGATTTTA GATTCTAAG GACAAGTCTT TTTAATCAAC CCAATGGTA TCACAATAGG TAAAGACGCA ATTATTAACA CTAATGGCTT
501 TACTGCTTCT ACCTAGACA TTTCTAAGCA AAACATCAAG GCGCGTAATT TCACCCTTGA GCAAAACCAAG GATAAAGCAC TCGTGAAAT CGTGAATCAC
601 GCTTTAATTA CCGTTGGTAA AGACGGTAGC GTAAACCTTA TTGGTGGCAA AGTGAAAAAC GAGGGCGTGA TTAGCGTAAA TGGCGGTAGT ATTTCTTTAC
701 TTGAGGGGCA AAAAATCACC ATCAGCGATA TAATAAATCC AACCATCACT TACAGCATTG CTGCACCTGA AAACGAAGCG ATCAATCTGG CGGATATTTT
801 TGCCAAAGGT GGTAAACATTA ATGTCCGGC TGCCACTATT CGCAATAAAG GTAAACTTTC TGCCGACTCT GTAAGCAAG ATAAAAGTGC TAACATTGTT
901 CTCTCTGCCA AAGAAGGTGA AGCGGAAATT GCGGGTAA TTCCGCTCA AATCAGCAA GCCAAAGGTG GTAAGTTGAT GATTACAGGC GATAAAGTTA
1001 CATTGAAAC GGGTGCGATT ATCGACCTTT CCGGTAAAGA AGGGGGAGAA ACTTATCTTG GCGGTGACGA GCGTGGCGAA GGTAAAAACG GCATTCAATT
1101 AGCAAAGAAA ACCACTTTAG AAAAAGGTC AACAAATTAAT GTGTGAGGTA AAGAAAAAGG TGGGGCGGCT ATTGTATGGG CGGATATTGC GTTAATTGAC
1201 GGCAATATTA ATGCCAAGG TAAAGATATC GCTAAACTG GTGGTTTTGT GGAGAGCTCG GGGCATTACT TATCCATTGA TGATAACGCA ATTGTTAAAA
1301 CAAAAGATG GCTACTAGAC CCAGAGAATG TGACTATTGA AGTCTCTCC GCTTCTCGCG TCGAGCTGGG TGCCGATAGG AATTCCTACT CGGAGAGGT
1401 GATAAAGTG ACCCTAAAA AAAATAACAC CTCCTTGACA AACTAACCA ATACAACCAT TTCAATCTT CTGAAAAGTG CCCACGTGGT GAACATAACG
1501 GCAAGGAGAA AACTACCGT TAATAGCTCT ATCAGTATAG AAAGAGGCTC CCCTTAATT CTCACAGTG AAGGTCAAGG CGGTCAAGGT GTTCAGATTG
1601 ATAAAGATAT TACTTCTGAA GCGGAAATT TAACCATTTA TTCTGGCGGA TGGGTTGATG TTGATAAAAA TATTACGCTT GGTAGCGGCT TTTTAAACAT
1701 CACAACTAAA GAAGGAGATA TCGCTTGA AGACAAGTCT GGACGGAA CACTAACCAT TACAGCCCA GGGACCATCA CCTCAGGTAA TAGTAACGGC
1801 TTTAGATTTA ACAACGTCTC TCTAACAGC CTTGGCGGAA AGCTGAGCTT TACTGACAGC AGAGAGGACA GAGGTAGAAG AACTAAGGGT AATATCTCAA
1901 ACAAAATTTGA CGGAACGTTA AACATTTCG GAACGTGAGA TATCTCAATG AAAGCACCCA AAGTCAGCTG GTTTTACAGA GACAAAGGAC GCACCTACTG
2001 GAACGTAACC ACTTTAAATG TTACCTCGG TAGTAAATTT AACCTCTCCA TTGACAGCAC AGGAAGTGGC TCAACAGGTC CAAGCATACG CAATGCAGAA
2101 TTAATGGCA TAACATTTAA TAAAGCCACT TTTAATATCG CACAAGGCTC AACAGCTAAC TTTAGCATCA AGGCATCAAT AATGCCCTTT AAGAGTAACG
2201 CTAACACGC ATTTATTAAT GAATATTTT CAGTCTCAGG GGGGGGTAGC GTTAATTTCA AACTTAACGC CTCATCTAGC AACATACAAA CCGCTGGCGT
2301 AATTATAAAA TCTCAAACT TTAATGTCTC AGGAGGGTCA ACTTTAAATC TCAAGGCTGA AGGTTCAACA GAAACCGCTT TTTCAATAGA AAATGATTTA
2401 AACTTAAACG CCACCGGTGG CAATATAACA ATCAGACAAG TCGAGGGTAC CGATTACCGC GTCAACAAAG GTGTCCGAGC CAAAAAAAC ATAACTTTTA
2501 AAGGGGGTAA TATCACCCTC GGTCTCAAA AAGCCACAAC AGAAATCAAA GGCAATGTTA CCATCAATAA AAACACTAAC GCTACTCTTC GTGGTGGGAA

46/73

2448

2601 TTTTGGCGAA AACAAATCGE CTTTAAATAT AGCAGGAAAT GTTATTAATA ATGGCAACCT TACCACTGCC GGCTCCATTA TCAATATAGC CGGAAATCTT
2701 ACTGTTTCAA AAGGCGCTAA CTTTCAAGCT ATAACAAATT ACACCTTTAA TGTAGCCGGC TCATTTGACA ACAATGGCGC TTCAAACATT TCCATTGGCA
2801 GAGGAGGGGC TAAATTTAAA GATATCAATA ACACCAGTAG CTTAAATATT ACCACCAACT CTGATACCAC TTACCGCACC ATTATAAAAG GCAATATATC
2901 CAACAAATCA GGTGATTGTA ATATTATTGA TAAAAAAGC GACCGTGAAA TCCAAATTGG CGGCAATATC TCACAAAAAG AAGGCAATCT CACAATTTCT
3001 TCTGATAAAG TAAATATTAC CAATCAGATA ACAATCAAAG CAGGCGTTGA AGGGGGGCGT TCTGATTCAA GTGAGGCAGA AAATGCTAAC CTAATATTCT
3101 AAACCAAAGA GTTAAATTTG GCAGGAGACC TAAATATTTC AGGCTTTAAT AAAGCAGAAA TTACAGCTAA AAATGGCAGT GATTTAACCTA TTGGCAATGC
3201 TAGCGGTGGT AATGCTGATG CTAATAAAGT GACTTTTGAC AAGGTTAAAG ATTCAAAAAT CTGACTGAC GGTCACAATG TAACACTAAA TAGCGAAGTG
3301 AAAACGTCTA ATGGTAGTAG CAATGCTGGT AATGATAACA GCACCGGTTT AACCATTTTC GCAAAAGATG TAACGGTAAA CAATAACGTT ACCTCCCA
3401 AGACAATAAA TATCTCTGCC GCAGCAGGAA ATGTAACAAC CAAAGAAGGC ACAACTATCA ATGCAACCAC AGGCAGCGTG GAAGTAACTG CTCAAAATGG
3501 TACAATTAAA GGCACATTA CCTCGCAAAA TGTAAACAGTG ACAGCAACAG AAAATCTTGT TACCACAGAG AATGCTGTCA TTAATGCAAC CAGCGGCA
3601 GTAAACATTA GTACAAAAAC AGGGGATATT AAAGGTGGAA TTGAATCAAC TTCCGGTAAT GTAAATATTA CAGCGAGCGG CAATACACTT AAGGTAAGTA
3701 ATATCACTGG TCAAGATGTA ACAGTAACAG CGGATGCAGG AGCCTTGACA ACTACAGCAG GCTCAACCAT TAGTGGCACA ACAGGCAATG CAAATATTAC
3801 AACCAAAACA GGTGATATCA ACGGTAAAGT TGAATCCAGC TCCGGCTCTG TAACACTTGT TGCAACTGGA GCAACTCTTG CTGTAGGTAA TATTTCAGGT
3901 AACACTGTTA CTATTACTGC GGATAGCGGT AAATTAACCT CCACAGTAGG TTCTACAATT AATGGGACTA ATAGTGTAAC CACCTCAAGC CAATCAGGCG
4001 ATATTGAAGG TACAATTTCT GGTAAATACAG TAAATGTTAC AGCAAGCACT GGTGATTTAA CTATTGGAAA TAGTGCAAAA GTTGAAGCGA AAAATGGAGC
4101 TGCAACCTTA ACTGCTGAAT CAGGCAAAAT AACCAACCAA ACAGGCTCTA GCATTACCTC AAGCAATGGT CAGACAACTC TTACAGCCAA GGATAGCAGT
4201 ATCGCAGGAA ACATTAATGC TGCTAATGTG ACGTTAAATA CCACAGGCAC TTAACTACT ACAGGGGATT CAAAGATTAA CGCAACCAGT GGTACCTTAA
4301 CAATCAATGC AAAAGATGCC AATTAGATG GTGCTGCATC AGGTGACCGC ACAGTAGTAA ATGCAACTAA CGCAAGTGGC TCTGGTAACG TCACTGCCAA
4401 AACCTCAAGC AGCGTGAATA TCACGGGGGA TTAAACACA ATAAATGGGT TAAATATCAT TTCGGAAAAT GGTAGAAACA CTGTGCGCTT AAGAGGCAAG
4501 GAAATTGATG TGAATATAT CCAACCAGGT GTAGCAAGCG TAGAAGAGGT AATTGAAGCG AAACGCGTCC TTCAGAAGGT AAAAGATTTA TCTGATGAAG
4601 AAAGAGAAAC ACTAGCCAAA CTTGGTGTA GGTGTGTAGG TTCTGTGAG CCAATAATG CCATTACGGT TAATACACAA AACGAGTTTA CAACCAACC
4701 ATCAAGTCAA GTGACAATTT CTGAAGGTAA GCGGTGTTT TCAAGTGTA ATGGCGCAGC AGTATGTACC AATGTTGCTG ACGATGGACA GCAG

47/73

Fin 9

HMW4 nucleotide sequence

Fin 9.1

REFORMAT of: Temp4.Gcg check: -1 from: 1 to: 4803 October 5, 1995 17:44

(No documentation)

Hmw4.Gcg Length: 4803 October 5, 1995 18:29 Type: M Check: 3920 ..

1 ATGAACAACA TATATCGTCT CAAATTCAGC AAACGCGTCA ATGCTTTGGT TGCTGTGTCT GAATTGACAC GGGGTTGTGA CCATTECACA GAAAAAGGCA
 101 GTGAAAAACC TGTTCGTACG AAGTACGCC ACTTGCGGT AAAGCCACTT TCCGTATAT TGCTATCTTT GGGCATGCA TCCATTCGCC AATCTGTTTT
 201 AGCGAGCGGT TTACAGGGAA TGAGCGTCT ACACGGTACA GCAACCATGC AAGTAGACGG CAATAAAACC ACTATCGTA ATAGCGTCAA TGCTATCATC
 301 AATTGGAAAC AATTTAATCAT TGACCAAAAT GAAATGGTGC AGTTTTTACA AGAAAGCAGC AACTCTGCGG TTTTCAACCG TGTTACATCT GACCAAACTC
 401 CCCAATTAAA AGGGATTTTA GATTCTAAGC GACAAGTCTT TTTAATCAAC CCAATGGTA TCACAATAGG TAAAGACGCA ATTATTAACA CTAATGGCTT
 501 TACTGCTTCT ACGETAGACA TTTCTAACGA AAACATCAAG GCGCGTAATT TCACCTTGA GCAAAACCAAG GATAAGCAC TCCTGAAAT CGTGAATCAC
 601 GGTTTAATTA CCGTTGGTAA AGACGGTAGC GTAAACCTTA TTGGTGGCAA AGTAAAAAC GAGGGCGTGA TTAGCGTAAA TGGCGGTAGT ATTTCTTTAC
 701 TTGCAGGGCA AAAAATCACC ATCAGCGATA TAATAAATCC AACCATCACT TACAGCATTG CTGCACCTGA AAACGAAGCG ATCAATCTGG GCGATATTTT
 801 TGCCAAAGGT GGTAACATTA ATGTCCGGCG TGCCACTATT CGCAATAAG GTAACTTTC TGCCACTCT GTAAGCAAG ATAAAGTGG TAACATGTT
 901 CTCTCTGCCA AAGAAGGTGA AGCGGAAAT GCGCGGTGA TTTCCGCTCA AATCAGCAA GCCAAAGGTG GTAAGTGTAT GATTACAGGT GATAAGTCA
 1001 CATTAAAAAC AGGTGCAGTT ATCGACCTTT CAGGTAAGA AGGGGGAGAG ACTTATCTTG GCGGTGATGA GCGTGGCGAA GGTAAAAATG GTATTCAATT
 1101 AGCGAAGAAA ACCTCTTTAG AAAAGGCTC GACAATTAAT GTATCAGCA AAGAAAAAGG CCGGGCGGCT ATTGTATGGG GCGATATTGC ATTAATTAAT
 1201 GGTAAACATTA ATGCTCAAGG TAGCGATATT GCTAAACTG GCGGCTTGT GGAACATCA GGACATGACT TATCCATTGG TGATGATGTG ATTTGTTGAGG
 1301 CTAAAGAGTG GTTATTAGAC CCAGATGATG TGTCCATTGA AACTCTTACA TCTGGACGCA ATAATACCGG CGAAAAACCA GGATATACAA CAGGAGATGG
 1401 GACTAAGAG TCACCTAAAG GTAATAGTAT TTCTAAACCT ACATTAACAA ACTCAACTCT TGAGCAATC CTAAGAAGAG GTTCTTATGT TAATATCACT
 1501 GCTAATAATA GAATTTATGT TAATAGCTCC ATCAACTTAT CTAATGGCAG TTTAACACTT CACACTAAAC GAGATGGAGT TAAATTAAC GGTGATATTA
 1601 CCTCAACGA AAATGGTAAT TTAACCATTA AAGCAGGCTC TTGGGTTGAT GTTCATAAAA ACATCAGCT TGGTACGGGT TTTTGAATA TTGTCGCTGG
 1701 GGATTCTGTA GCTTTTGAGA GAGAGGGCGA TAAAGCAGT AACGCAACAG ATGCTCAAT TACCGCACAA GGCAGGATAA CCGTCAATAA AGATGATAAA
 1801 CAATTTAGAT TCAATAATGT ATCTATTAAC GGGACGGGCA AGGGTTTAA GTTTATTGCA AATCAAAATA ATTTCACTCA TAAATTTGAT GCGGAAATTA
 1901 ACATATCTGG AATAGTAACA ATTAACCAAA CCACGAAAAA AGATGTTAAA TACTGGAATG CATCAAAAGA CTCTTACTGG AATGTTTCTT CTCTTACTTT
 2001 GAATACGGTG CAAAAATTTA CTTTATAAAA ATTCGTTGAT AGCGGCTCAA ATTCCTCAAG TTTGAGGTCA TCACGTAGAA GTTTTGACAG CGTACATTTT
 2101 AACGGCATCG GAGGCAAAAC AAATTCAAC ATCGGAGCTA ACGCAAAAGC CTTATTTAAA TTAACCAAA ACGCGCTAC AGACCCAAAA AAAGAATTAC
 2201 CTATTACTTT TAACGCCAAC ATTACAGCTA CCGGTAACAG TGATAGCTCT GTGATGTTG ACATACACCG CAATCTTACC TCTAGAGCTG CCGGCATAAA
 2301 CATGGATTCA ATTAACATTA CCGGCGGCT TGACTTTTCC ATACATCCC ATAATCGCAA TAGTAATGCT TTTGAAATCA AAAAGACTT AACTATAAAT
 2401 GCACTGGCT CGAATTTTAG TCTTAAGCAA ACGAAAGATT CTTTATATAA TGAATACAGC AAACACGCCA TTAATCAAG TCATAATCTA ACCATTCTTG

48/73

27 98

2501 GCGGCAATGT CACTCTAGGT GGGGAAAATT CAAGCAGTAG CATTACGGGC AATATCAATA TCACCAATAA AGCAATGTT ACATTACAAG CTGACACCAG
2601 CAACAGCAAC ACAGGCTTGA AGAAAAGAAC TCTAECTTTT GGCAATATAT CTGTTGAGGG GAATTTAAGC CTAAGTGGTG CAAATGCAAA CATTGTCGGC
2701 AATCTTTCTA TTGCAGAAGA TTCCACATTT AAAGGAGAAG CCAGTGACAA CCTAAACATC ACCGGGACCT TTACCAACAA CGGTACCGCC AACATTAATA
2801 TAAACAAGG AGTGGTAAAA CTCCAAGGCG ATATTATCAA TAAAGGTGGT TTAATATCA CTAATAACGC CTCAGGCACT CAAAAACCA TTATTAACGG
2901 AAATATACT AACGAAAAAG GCGACTTAAA CATCAAGAAT ATTAAGGCGC ACGCCGAAAT CCAATTGGC GGCAATATCT CAAAAAAGA AGGCAATCTC
3001 ACAATTTCTT CTGATAAAGT AAATATTACC AATCAGATAA CAATCAAAGC AGCGGTGAA GGGGGGCGTT CTGATTCAAG TGAGGCAGAA AATGCTAACC
3101 TAACTATTCA AACCAAGAGC TTAAAATTGG CAGGAGACCT AAATATTCA GGCTTTAATA AAGCAGAAAT TACAGCTAAA AATGGCAGTG ATTTAACTAT
3201 TGGCAATGCT AGCGGTGGTA ATGCTGATGC TAAAAAGTG ACTTTTGACA AGGTTAAAGA TTCAAAAATC TCGACTGAGG GTCACAATGT AACACTAAAT
3301 AGCGAAGTGA AAACGTCTAA TGGTAGTAGC AATGCTGGTA ATGATAACAG CACCGGTTTA ACCATTTCCG CAAAGATGT AACGGTAAAC AATAACGTTA
3401 CTTCCACAA GACAATAAAT ATCTCTGCCG CAGCAGGAAA TGAACAACC AAAGAAGGCA CAACTATCAA TGCAACCACA GGCAGCGTGG AAGTAACTGC
3501 TCAAAATGGT ACAATTAAAG GCAACATTAC CTCGCAAAAT GTACAGTGA CAGCAACAGA AAATCTTGT ACCACAGAGA ATGCTGTCTAT TAATGCAACC
3601 AGCGGCACAG TAAACATTAG TACAAAAACA GGGGATATTA AAGGTGGAAT TGAATCACT TCCGGTAATG TAAATATTAC AGCGAGCGGC AATACACTTA
3701 AGGTAAGTAA TATCACTGGT CAAGATGTAA CAGTAACAGC GGATGCAGGA GCCTTGACAA CTACAGCAGG CTCACCATT AGTGGGACAA CAGGCAATGC
3801 AAATATTACA ACCAAAACAG GTGATATCAA CGGTAAAGTT GAATCCAGCT CCGGCTCTGT AACACTTGT GCAACTGGAG CAACTCTTGC TGTAGGTAAT
3901 ATTTGAGGTA AACTGTGTAC TATTACTGCG GATAGCGGTA AATTAACCTC CACAGTAGGT TCTACAATTA ATGGGACTAA TAGTGTAACC ACCTCAAGCC
4001 AATCAGGCGA TATTGAAGGT ACAATTTCTG GTAATACAGT AAATGTTACA GCAAGCACTG GTGATTTAAC TATTGGAAT AGTGCAAAAG TTGAAGCGAA
4101 AAATGGAGCT GCAACCTTAA CTGCTGAATC AGGCAATTA ACCACCCAAA CAGGCTCTAG CATTACCTCA AGCAATGGTC AGACAACCTCT TACAGCCAAG
4201 GATAGCAGTA TCGCAGGAAA CATTAATGCT GCTAATGTGA CGTTAAATAC CACAGGCACT TTAAGTACTA CAGGGGATTC AAAGATTAA GCAACCAGTG
4301 GTACCTTAA AATCAATGCA AAAGATGCCA AATTAGATGG TGCTGCATCA GGTGACCGCA CAGTAGTAAA TGCAACTAAC GCAAGTGGCT CTGGTAACGT
4401 GACTGCGAAA ACCTCAAGCA GCGTGAATAT CACCGGGGAT TTAACACAA TAAATGGGT AAATATCATT TCGGAAAATG GTAGAAACAC TGTCGGCTTA
4501 AGAGGCAAGG AAATGATGT GAAATATATC CAACCAGGTG TAGCAAGCGT AGAAGAGGTA ATTGAAGCGA AACCGCTCT TGAGAAAGTA AAAGATTTAT
4601 CTGATGAAGA AAGAGAAACA CTAGCCAAAC TTGGTGTAG TGCTGTACGT TTCGTTGAGC CAAATAATGC CATTACGGTT AATACACAAA ACGAGTTTAC
4701 AACCAACCA TCAAGTCAAG TGACAATTTC TGAAGGTAAG GCGTGTCTCT CAAGTGGTAA TGGCGCACGA GTATGTACCA ATGTTGCTCA CGATGGACAG
4801 CAG

FIG. 10A. COMPARISON OF DERIVED AMINO ACID SEQUENCE

	1								50
Hmw3com	M N K I Y R L K F S	K R L N A L V A V S	E L A R G C D H S T	E K G S E K P A R M	K V R H L A L K P L				
Hmw4com	M N K I Y R L K F S	K R L N A L V A V S	E L A R G C D H S T	E K G S E K P A R M	K V R H L A L K P L				
Hmw1com	M N K I Y R L K F S	K R L N A L V A V S	E L A R G C D H S T	E K G S E K P A R M	K V R H L A L K P L				
Hmw2com	M N K I Y R L K F S	K R L N A L V A V S	E L A R G C D H S T	E K G S E K P A R M	K V R H L A L K P L				
	51								100
Hmw3com	S A I L L S L G M A	S I P Q S V L A S G	L Q G M S V V H G T	A T M Q V D G N K T	T I R N S V N A I I				
Hmw4com	S A I L L S L G M A	S I P Q S V L A S G	L Q G M S V V H G T	A T M Q V D G N K T	T I R N S V N A I I				
Hmw1com	S A M L L S L G V T	S I P Q S V L A S G	L Q G M S V V H G T	A T M Q V D G N K T	T I R N S V N A I I				
Hmw2com	S A M L L S L G V T	S I P Q S V L A S G	L Q G M S V V H G T	A T M Q V D G N K T	T I R N S V N A I I				
	101								150
Hmw3com	N W K Q F N I D Q N	E M E Q F L Q E S S	N S A V F N R V T S	D Q I S Q L K G I L	D S N G Q V F L I N				
Hmw4com	N W K Q F N I D Q N	E M E Q F L Q E S S	N S A V F N R V T S	D Q I S Q L K G I L	D S N G Q V F L I N				

49/73

50/73

Hmw1.com	NWKQFNIDQN	EMVQFLQENN	NSAVFNRVTS	NQISQLKGIL	DSNGQVFLIN
Hmw2.com	NWKQFNIDQN	EMVQFLQENN	NSAVFNRVTS	NQISQLKGIL	DSNGQVFLIN

151
200

Hmw3com	Pn{i1i5kda j1n1n5ftas jld1sn1en1k ar~ftleqtk dkala1e1vnH
Hmw4com	PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH
Hmw1com	PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH
Hmw2com	PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH

201

250

Hmw3com	5LITV5KDGS	VNLJ55KVK	Q5VIVN555	JSLCA9QKIT	ISDIINPTIT
Hmw4com	GLITVGKDG	VNLGGKVK	EGVISVNGG	ISLLAGQKIT	ISDIINPTIT
Hmw1com	GLITVGKDG	VNLGGKVK	EGVISVNGG	ISLLAGQKIT	ISDIINPTIT
Hmw2com	GLITVGKDG	VNLGGKVK	EGVISVNGG	ISLLAGQKIT	ISDIINPTIT

251 300

Htmw3com
 ;SIAPEEA
 INLGDIKAG GNINVRAATI RNKGKLSADS VSKDKSGNIV

51/73

FIG. 10D.

Hmw4com YSIAAPENEA INLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV
Hmw1com YSIAAPENEA VNLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV
Hmw2com YSIAAPENEA VNLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV

301

350

Hmw3com LSAKEGEAEI GGVisAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
Hmw4com LSAKEGEAEI GGVisAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
Hmw1com LSAKEGEAEI GGVisAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
Hmw2com LSAKEGEAEI GGVisAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE

351

400

Hmw3com TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
Hmw4com TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
Hmw1com TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
Hmw2com TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID

FIG. 10E.

	401	450
Hmw3com	GNINAQK.D IAKTGGFVET	SGHYLSIDDN AIVKKEWLL DPENVTEAP
Hmw4com	GNINAQGS.D IAKTGGFVET	SGHDLSIGDD VIVDAKEWLL DPDDVSIETL
Hmw1com	GNINAQGS.D IAKTGGFVET	SGHDLFIKDN AIVDAKEWLL DPDNV Tinae
Hmw2com	GNINAQGS.D IAKTGGFVET	SGHYLSIESN AIVKKEWLL DPDDVTIEAE
	451	500
Hmw3com	SASRVELGAD RNHSAEVIK	VTLKKNNTSL TTLTNTTISN LLKSAHVVNI
Hmw4com	TSGRNNNTGEN QGYTTGDGK	ESPKGNSISK PTLTNTSTLEQ ILRRGSYVNI
Hmw1com	TAGRSNTSED DEYTGSGNSA	STPKRKE.K TTLTNTTTLES ILKKGTFVNI
Hmw2com	DPLRNNNTGIN DEFPTGTGEA	SDPKKNSELK TTLTNTTISN YLKNAWTMNI
	501	550
Hmw3com	TARRKLTVNS SIERGSHL	ILHSEGQGGQ GVQIDKDITS .E...GGNLT
Hmw4com	TANNRIYVNS SINLSNGS.L	TLHTK...RD GVKINGDITS NE...NGNLT
Hmw1com	TANQRIYVNS SINL.SNGSL	TLWSEGRSGG GVEINNDDITT GDDTRGANLT
Hmw2com	TASRKLTVNS SINGSNHSL	ILHSGQRRG GVQIDGDIT. ...SKGGNLT

52/73

59/73

FIG. 10F.

551
 Hmw3com IYSGGWVDVH KNITLGS.GF LNITTKEGDI AFEDKSGR...NNLTITAQ 600
 Hmw4com IKAGSWVDVH KNITLGT.GF LNIVAGDS.V AFEREGDKAR NATDAQITAQ
 Hmw1com IYSGGWVDVH KNISLGAQGN INITAKQD.I AFEKGSNQV.ITGQ
 Hmw2com IYSGGWVDVH KNITLD.QGF LNITA.AS.V AFEKGNNKAR DANNLTITAQ

601
 Hmw3com GTITSG.NSN GFRFNNVSLN SLGGKLSFTD SREDRGRRTK GNISNKFDDGT 650
 Hmw4com GTITVKNKDDK QFRFNNVSLN GTGKGLKFIA NQN.....NFTHKFDGE
 Hmw1com GTIT.SGNQK GFRFNNVSLN GTGSGLQFTT KRTN.....K YAITNKFEGT
 Hmw2com GTVTITGEGK DFRANNVSLN GTGKGLNIIS SVNN.....LTHNLSGT

651
 Hmw3com LNISGTVDIS MKAPKVSIFY RD.KGRTYWN VTTLNVTSGS KFNLSIDSTG 700
 Hmw4com INISGIVTIN QTTKKDKVKYW NA.SKDSYWN VSSLTLNTVQ KFTF.IKFVD
 Hmw1com LNISGKVNIS MVLPKNESGY DKFKGRTYWN LTSLNVSESG EFNLTIDSRG

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FIG. 10G.

Hmw2com INISGNITIN QTTRKNTSYW QTSHD..SHWN VSALNLETGA NTF. IKYIS

701 750

Hmw3com SGSTG...PS IRNA..ELNG ITFN....KA TFNIAQGSTA NFSIKASIMP

Hmw4com SGSNS...QD LRSSRRSFAG VHFNGIGGKT NFNIGANAKA LFKLKPNAAT

Hmw1com SDSAGTLTQ.PYNLNG ISFN...KDT TENVERNARV NFDIKAPIGI

Hmw2com SNSKGLTTQY RSSAGVNFNG V..N...GNM SFNLKEGAKV NFKLKPENNM

751

54/2 800

Hmw3com FKSANYAL. FNEDISVG. .GGSVNFKN ASSNIQTPG VIKSQNFNV

Hmw4com DPKKELPIT. FNANITATGN SDSSVMFDIH A...NLTSRA AGINMDSINI

Hmw1com NKYSSLNYAS FNGNISVG. .GGSVDFTL ASSNVQTPG VVINSKYFNV

Hmw2com NTSKPLPI.R FLANITATG. .GGSVFFDIY ANHS...GRG AELKMSEINI

801

850

Hmw3com SGGSTLNLKA EGSTETAFSI ENDLNLNATG GNITIRQVEG T..DSRVNKG

Hmw4com TGGLDFSITS HNRNSNAFEI KKDLTINATG SNFSLKQTKD SFYNEYSKHA

RECTIFIED SHEET (RULE 91)

FIG. 10H.

Hmw1com STGSSLRFKT SGSTKTGFSI EKDLTLNATG GNITLLQVEG T..DGMIGKG
 Hmw2com SNGANFTLNS HVRGDDAFKI NKDLTINATN SNFSLRQTKD DFYDGYARNA

851

900

Hmw3com VAAKKNITFK GGNITFGSQK ATTEIKGNVT INKNTNATLR GANFAEN...
 Hmw4com INSSHNLTL GGNVTLGGEN SSSITGNIN ITNKANVTLQ ADTSNSNTGL
 Hmw1com IVAKKNITFE GGNITFGSRK AVTEIEGNVT INNNANVTLI GSDFDNHQ..
 Hmw2com INSTYNISIL GGNVTLGGQN SSSITGNIT IEKAAANVTLE ANNAPNQONI

55/23

901

950

Hmw3com KSPLNIAGNV INNGNLTTAG SIINIAGNLT VSKGANLQAI TNYTFNVAGS
 Hmw4com KKRTLTLGNI SVEGNLSLTG ANANIVGNLS IAEDSTFKGE ASDNLNITGT
 Hmw1com KPLTIKKDVI INSGNLTAGG NIVNIAGNLT VESNANFKAI TNFTFNVGGL
 Hmw2com RDRVIKLGSL LVNGSLSLTG ENADIKGNLT ISESATFKGK TRDTLNLITGN

951

1000

RECTIFIED SHEET (RULE 91)

56/73

FIG. 10I.

Hmw3 com	FDNNGASNIS	IARGGAKFK.	DINNTSSLNI	TTNSDTTYRT	IIKGNISNKS
Hmw4 com	FTNNGTANIN	IKQGVVKLQG	DINNKGGLNI	TTNASGTQKT	IINGNITNEK
Hmw1 com	FDNKGNSNIS	IAKGGARFK.	DIDNSKNLSI	TTNSSSTYRT	IISGNITNKN
Hmw2 com	FTNNGTAEIN	ITQGVVKLG.	NVTNDGDLNI	TTHAKRNQRS	IIGGDIINN

1001

1050

Hmw3 com	GDLNIIDKKS	DAEIQIGGNI	SQKEGNLTIS	SDKVNITNQI	TIKAGVEGGR
Hmw4 com	GDLNIKNIKA	DAEIQIGGNI	SQKEGNLTIS	SDKVNITNQI	TIKAGVEGGR
Hmw1 com	GDLNITNEGS	DTEMQIGGDI	SQKEGNLTIS	SDKINITKQI	TIKAGVDGEN
Hmw2 com	GSLNITDSNN	DAEIQIGGNI	SQKEGNLTIS	SDKINITKQI	TIKKGIDGED

1051

1100

Hmw3 com	SDSSEAENAN	LTIQTKELKL	AGDLNISGFN	KAEITAKNGS	DLTIGNASGG
Hmw4 com	SDSSEAENAN	LTIQTKELKL	AGDLNISGFN	KAEITAKNGS	DLTIGNASGG
Hmw1 com	SDSDATNNAN	LTIKTKELKL	TQDLNISGFN	KAEITAKDGS	DLTIGNTNSA
Hmw2 com	SSSDATSNAN	LTIKTKELKL	TEDLSISGFN	KAEITAKDGR	DLTIGNSNDG

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FIG. 10J.

	1101		1150
Hmw3com	N..ADAKKVT	FDKVKDSKIS	TDGHNVTLNS EVKT..SNGS SNAGNDNSTG
Hmw4com	N..ADAKKVT	FDKVKDSKIS	TDGHNVTLNS EVKT..SNGS SNAGNDNSTG
Hmw1com	D.GTNAKKVT	FNQVKDSKIS	ADGHKVTLIIS KVETSGSNNN TEDSSDNNAG
Hmw2com	NSGAEAKKVT	FNNVKDSKIS	ADGHNVTLNS KVKTSSSNGG RESNSDNDTG
	1151		1200
Hmw3com	LTISAKDVTV	NNNVTSHKTI	NISAAAGNVT TKEGTTINAT TGSVEVTAQN
Hmw4com	LTISAKDVTV	NNNVTSHKTI	NISAAAGNVT TKEGTTINAT TGSVEVTAQN
Hmw1com	LTIDAKNVTV	NNNITSHKAV	SISATSGEIT TKTGTTINAT TGNVEIT...
Hmw2com	LTITAKNVEV	NKDVTSLKTV	NITA.SEKVT TTAGSTINAT NGKASIT...
	1201		1250
Hmw3com	GTIKGNITSQ	NVTVTATENL	VTTENAVINA TSGTVNISTK TGDIKGGIES
Hmw4com	GTIKGNITSQ	NVTVTATENL	VTTENAVINA TSGTVNISTK TGDIKGGIES
Hmw1comAQ TGDIKGGIES

57/23

FIG. 10K.

Hmw2comTK T.....

1251 1300
Hmw3com TSGNVNITAS GNTLKVSNIT GQDVTVTADA GALTITAGST ISATTGNANI
Hmw4com TSGNVNITAS GNTLKVSNIT GQDVTVTADA GALTITAGST ISATTGNANI
Hmw1com SSGSVTLTAT EGALAVSNIS GNTVTVTANS GALTITAGST IKG.TESVTT
Hmw2com

1301 1350
Hmw3com TTKTGDKNGK VESSSGSVTL VATGATLAVG NISGNTVTIT ADGKLTSTV
Hmw4com TTKTGDKNGK VESSSGSVTL VATGATLAVG NISGNTVTIT ADGKLTSTV
Hmw1com SSQSGDIG..G TISGGTVEVK ATESLTTQSN
Hmw2comGDIS..G TISGNTVSVS ATVDLTTKSG

1351 1400
Hmw3com GSTINGTNSV TTSSQSGDIE GTISGNTVNV TASTGDLTIG NSAKVEAKNG
Hmw4com GSTINGTNSV TTSSQSGDIE GTISGNTVNV TASTGDLTIG NSAKVEAKNG

59/73

FIG. 10L.

Hmw1com SKIKATTGEA NVTSATGTIG GTISGNTVNV TANAGDLTVG NGAEINATEG
 Hmw2com SKIEAKSGEA NVTSATGTIG GTISGNTVNV TANAGDLTVG NGAEINATEG

1401 1450
 Hmw3com AATLTAESGK LTTQTGSSIT SSNGQTTTLTA KDSSIAGNIN AANVTLNNTTG
 Hmw4com AATLTAESGK LTTQTGSSIT SSNGQTTTLTA KDSSIAGNIN AANVTLNNTTG
 Hmw1com AATLTTSSGK LTTEASSHIT SAKGQVNLSA QDSSVAGSIN AANVTLNNTTG
 Hmw2com AATLTATGNT LTTEAGSSIT STKGQVDLLA QNSSIAGNIN AANVTLNNTTG

1451 1500
 Hmw3com TLTTTGDSKI NATSGTLTIN AKDAKLDGAA SGDRTVVNAT NASGSGNVTA
 Hmw4com TLTTTGDSKI NATSGTLTIN AKDAKLDGAA SGDRTVVNAT NASGSGNVTA
 Hmw1com TLTTVKGSNI NATSGTLTIN AKDAELNGAA LGNHTVVNAT NANGSGSVIA
 Hmw2com TLTTVAGSDI KATSGTLTIN AKDAKLNDA SGDSTEVENAV NASGSGSVTA

1501 1550

RECTIFIED SHEET (RULE 91)

FIG. 10L.

Hmw3com KTSSSVNITG DLNTINGLNI ISENGRNTVR LRGKEIDVKY IQPGVASVEE
 Hmw4com KTSSSVNITG DLNTINGLNI ISENGRNTVR LRGKEIDVKY IQPGVASVEE
 Hmw1com TTSSRVNITG DLITINGLNI ISKNGINTVL LKGVKIDVKY IQPGIASVDE
 Hmw2com ATSSSVNITG DLNTVNGLNI ISKDGRTVR LRGKEIEVKY IQPGVASVEE

1551

1600

Hmw3com VIEAKRVLEK VKDLSDEERE TLAKLGVS AV RFVEPNNAIT VNTQNEFTTK
 Hmw4com VIEAKRVLEK VKDLSDEERE TLAKLGVS AV RFVEPNNAIT VNTQNEFTTK
 Hmw1com VIEAKRILEK VKDLSDEERE ALAKLGVS AV RFIEPNNTIT VDTQNEFATR
 Hmw2com VIEAKRVLEK VKDLSDEERE TLAKLGVS AV RFVEPNNTIT VNTQNEFTTR

60/73

1601

1632

Hmw3com PSSQVTISEG KACFSSGNGA RVCTNVADDG QQ (1st 10 ~ 9)
 Hmw4com PSSQVTISEG KACFSSGNGA RVCTNVADDG QQ (1st 10 ~ 10)
 Hmw1com PLSRIVISEG RACFSNSDGA TVCVNIADNG R. (1st 10 ~ 2)
 Hmw2com PSSQVIISEG KACFSSGNGA RVCTNVADDG QP (1st 10 ~ 4)

61 / 73

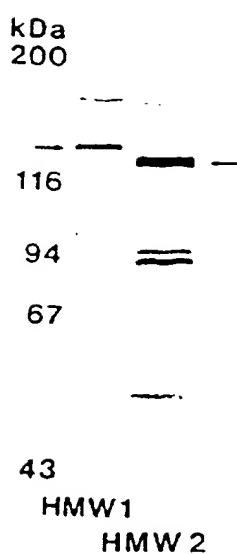
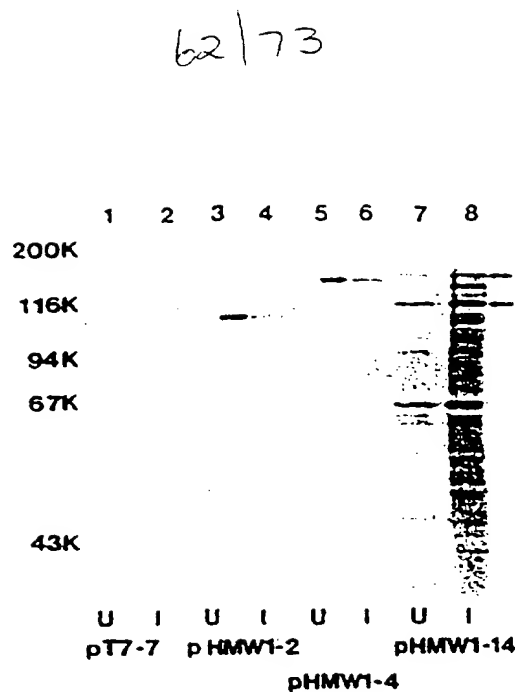


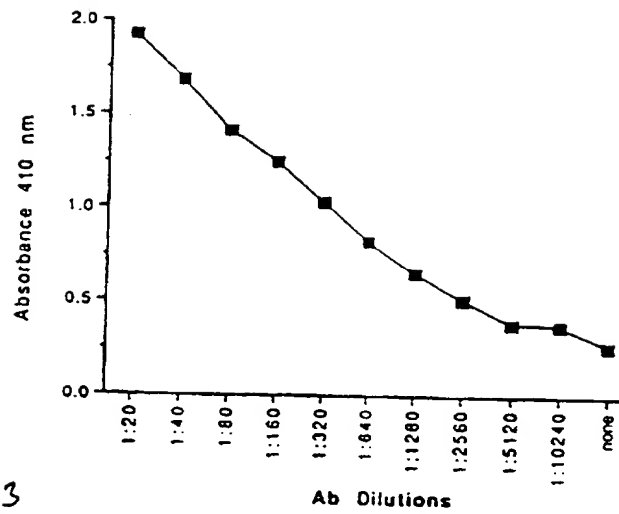
FIG. 2. Western immunoblot assay of phage lysates containing either the HMW1 or HMW2 recombinant proteins. Lysates were probed with an *E. coli*-absorbed adult serum sample with high-titer antibody against high-molecular-weight proteins. The arrows indicate the major immunoreactive protein bands of 125 and 120 kDa in the HMW1 and HMW2 lysates, respectively.



12

FIG. 3. Western immunoblot assay of cell sonicates prepared from *E. coli* transformed with plasmid pT7-7 (lanes 1 and 2), pHMW1-2 (lanes 3 and 4), pHMW1-4 (lanes 5 and 6), or pHMW1-14 (lanes 7 and 8). The sonicates were probed with an *E. coli*-absorbed adult serum sample with high-titer antibody against high-molecular-weight proteins. Lanes labeled U and I represent sonicates prepared before and after induction of the growing samples with IPTG, respectively. The arrows indicate protein bands of interest as described in the text.

63/73



13

FIG. 6. ELISA with rHMW1 antiserum assayed against purified filamentous hemagglutinin of *B. pertussis*. Ab, antibody.

64/73

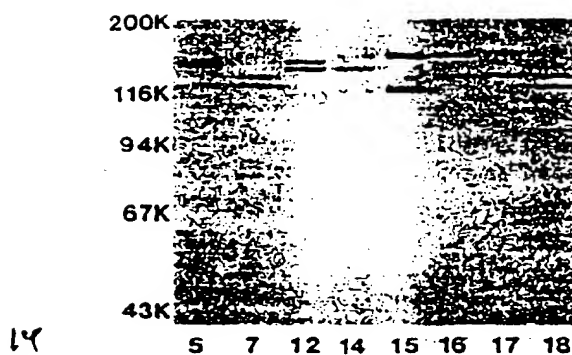
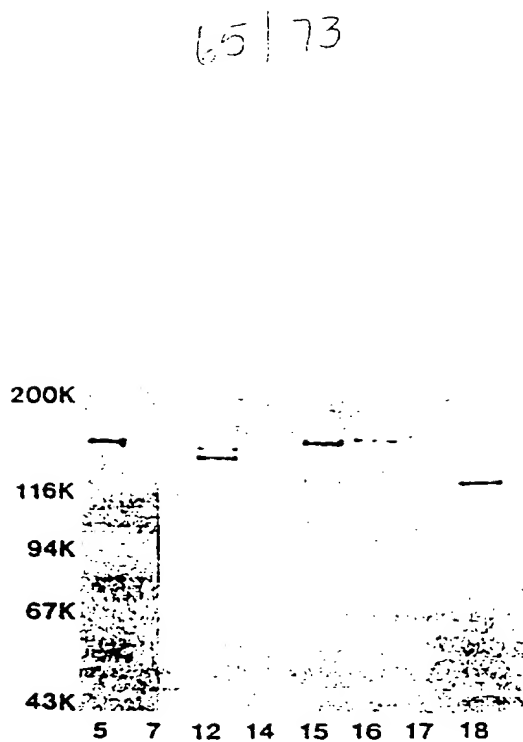
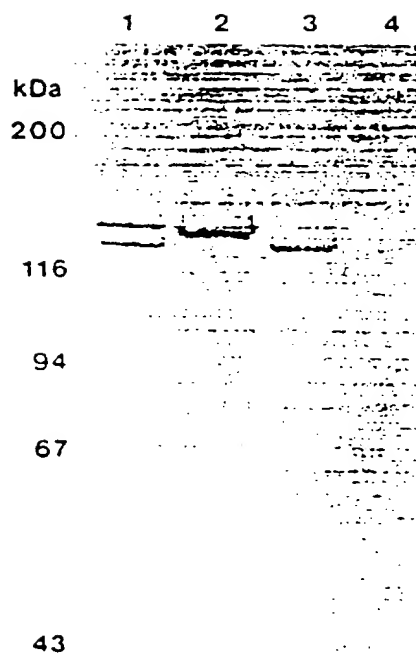


FIG. 7. Western immunoblot assay of cell sonicates from a panel of epidemiologically unrelated nontypeable *H. influenzae* strains. The sonicates were probed with rabbit antiserum prepared against HMW1-4 recombinant protein. The strain designations are indicated by the numbers below each lane.



15
FIG. 8. Western immunoblot assay of cell sonicates from a panel of epidemiologically unrelated nontypeable *H. influenzae* strains. The sonicates were probed with monoclonal antibody X3C, a murine IgG antibody which recognizes the filamentous hemagglutinin of *B. pertussis* (13). The strain designations are indicated by the numbers below each lane.

66/73



16
FIG. 2. Immunoblot assay of cell sonicates of nontypable *H. influenzae* strain 12 derivatives. The sonicates were probed with rabbit antiserum prepared against HMW-1 recombinant protein. Lanes: 1, wild-type strain; 2, HMW-2⁻ mutant; 3, HMW-1⁻ mutant; 4, HMW-1⁻/HMW-2⁻ double mutant.

6/7/73

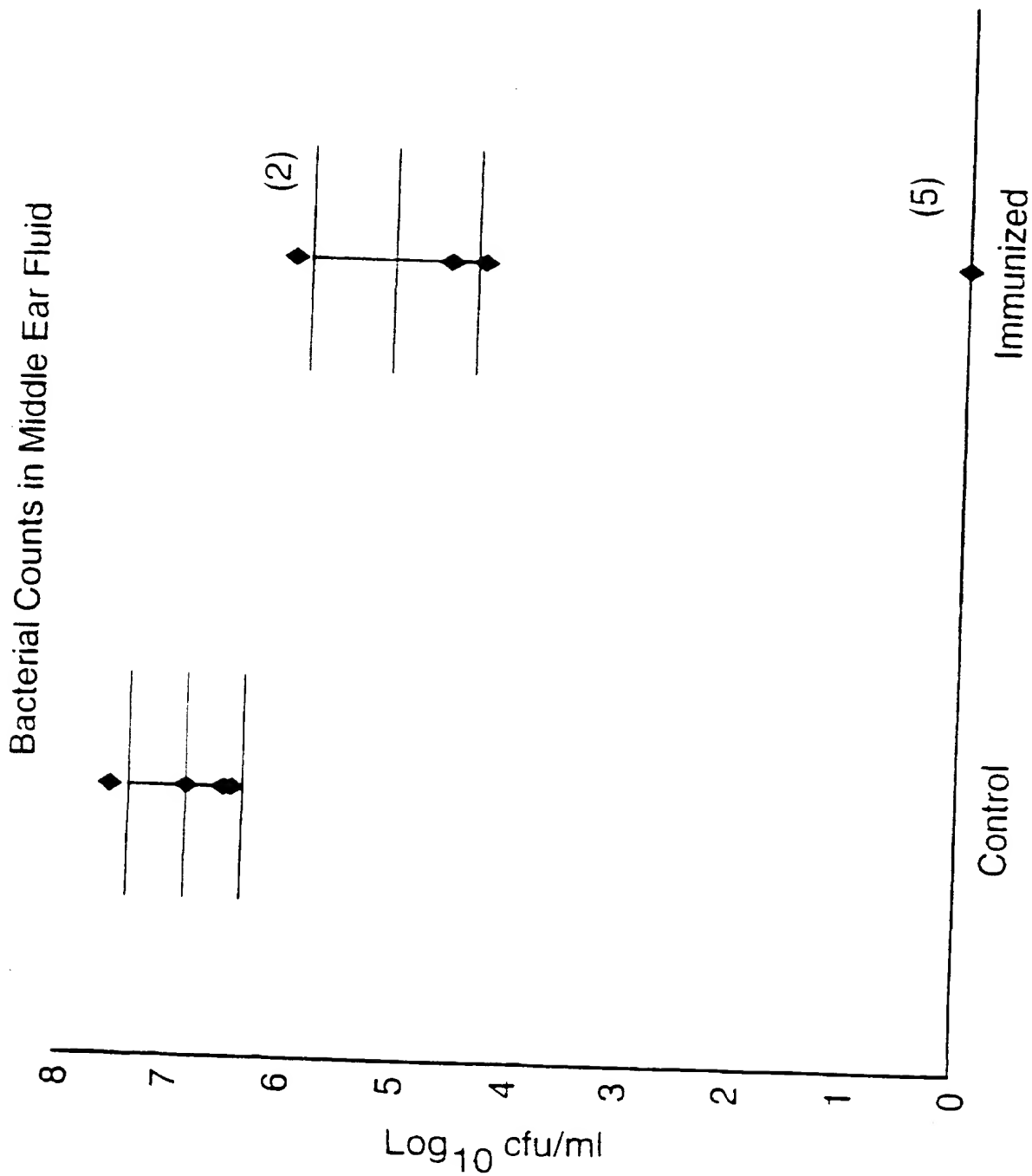


FIG.17

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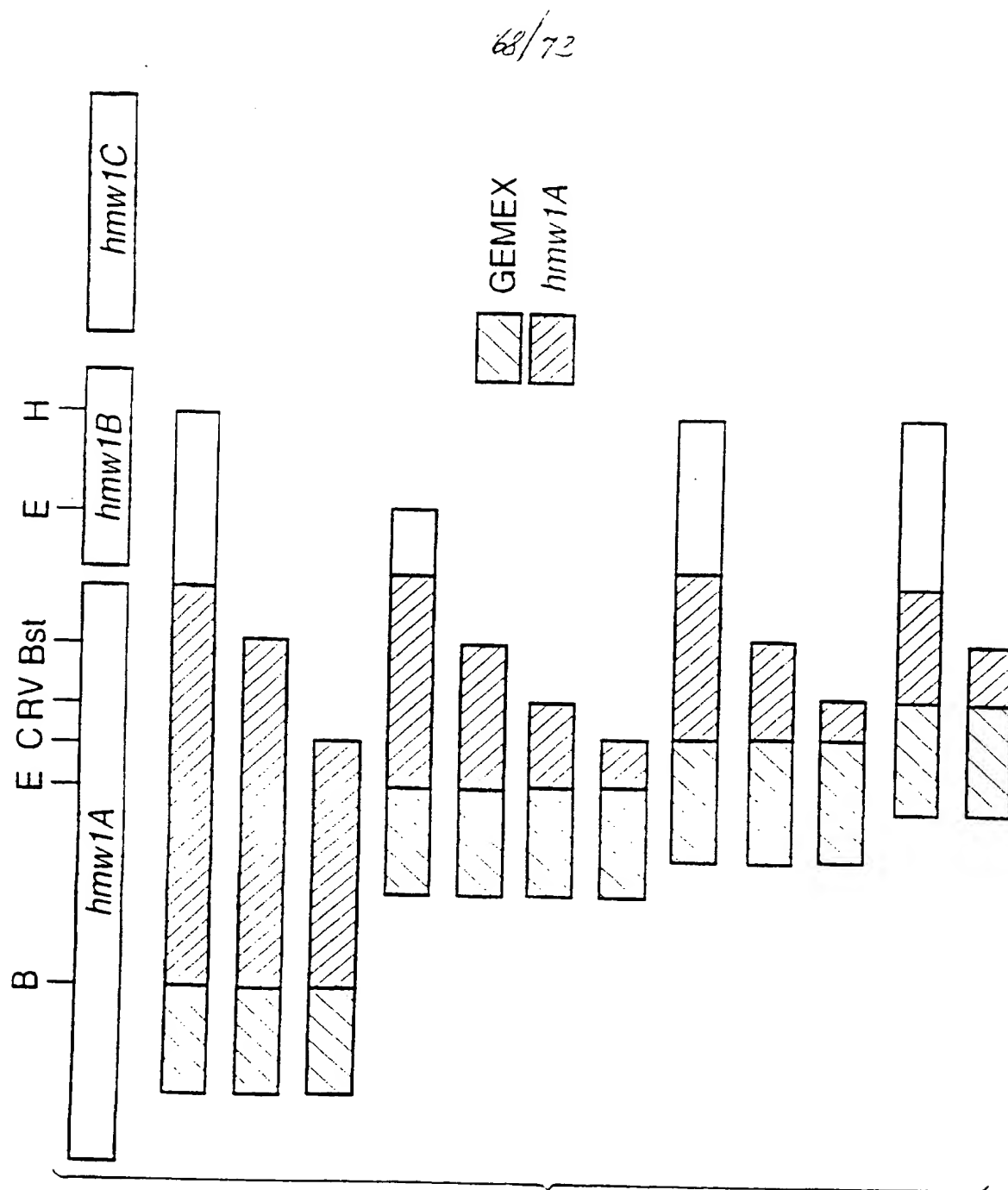


FIG.18

69/73

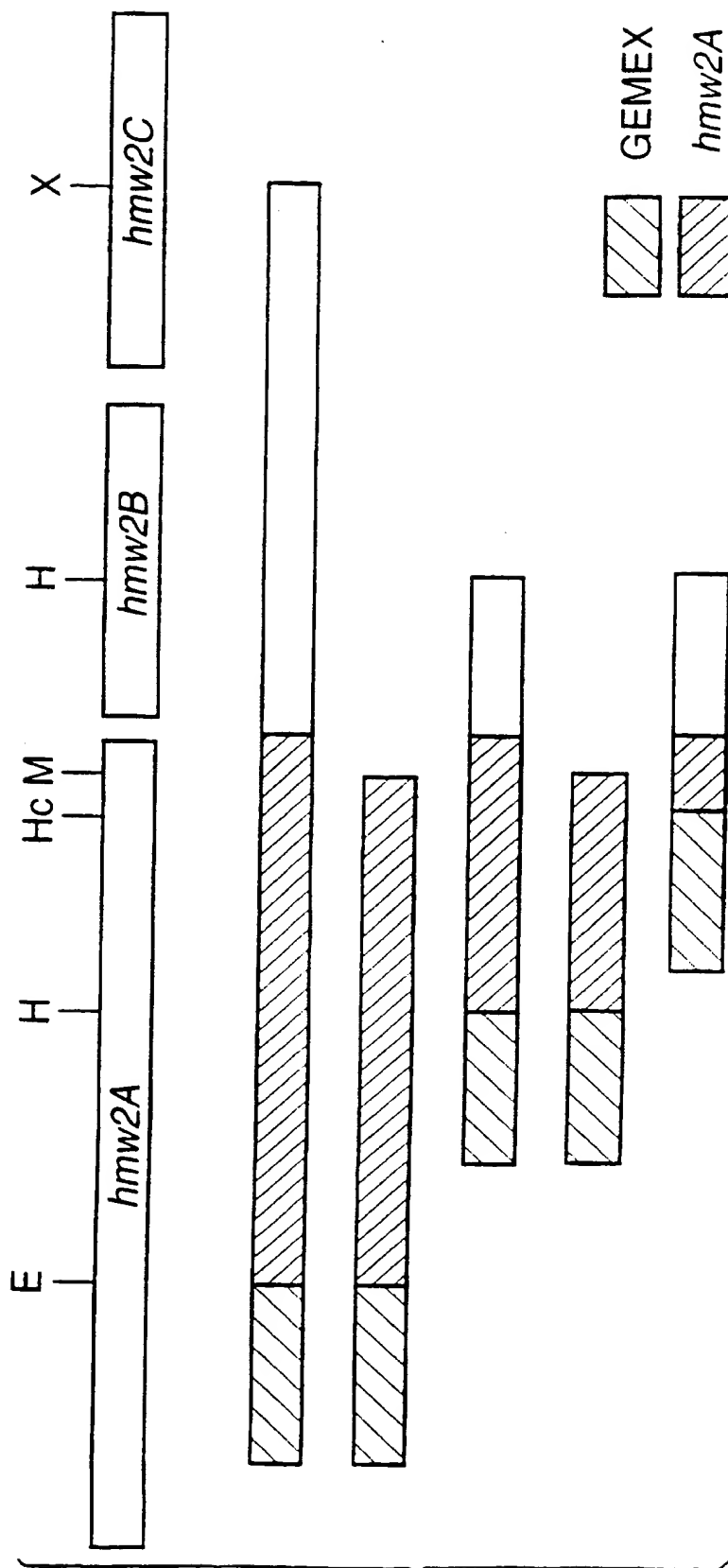


FIG.19

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70/73

Immunoelectron microscopy with Mab AD6

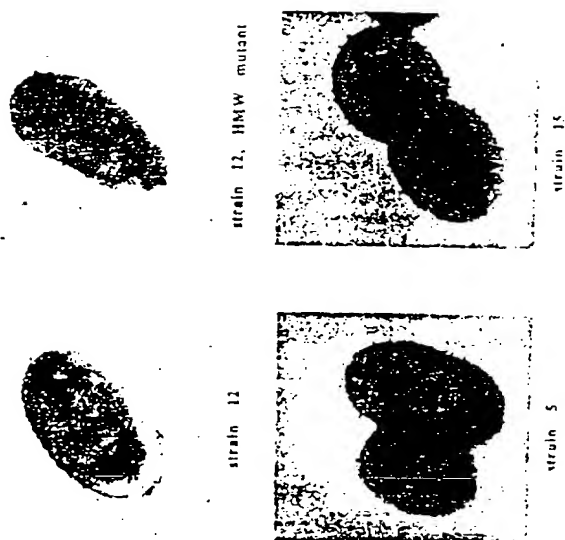


Figure 20

71 | 73

Western immunoblot assay with Mab AD6 and
HMW1A or HMW2A recombinant proteins

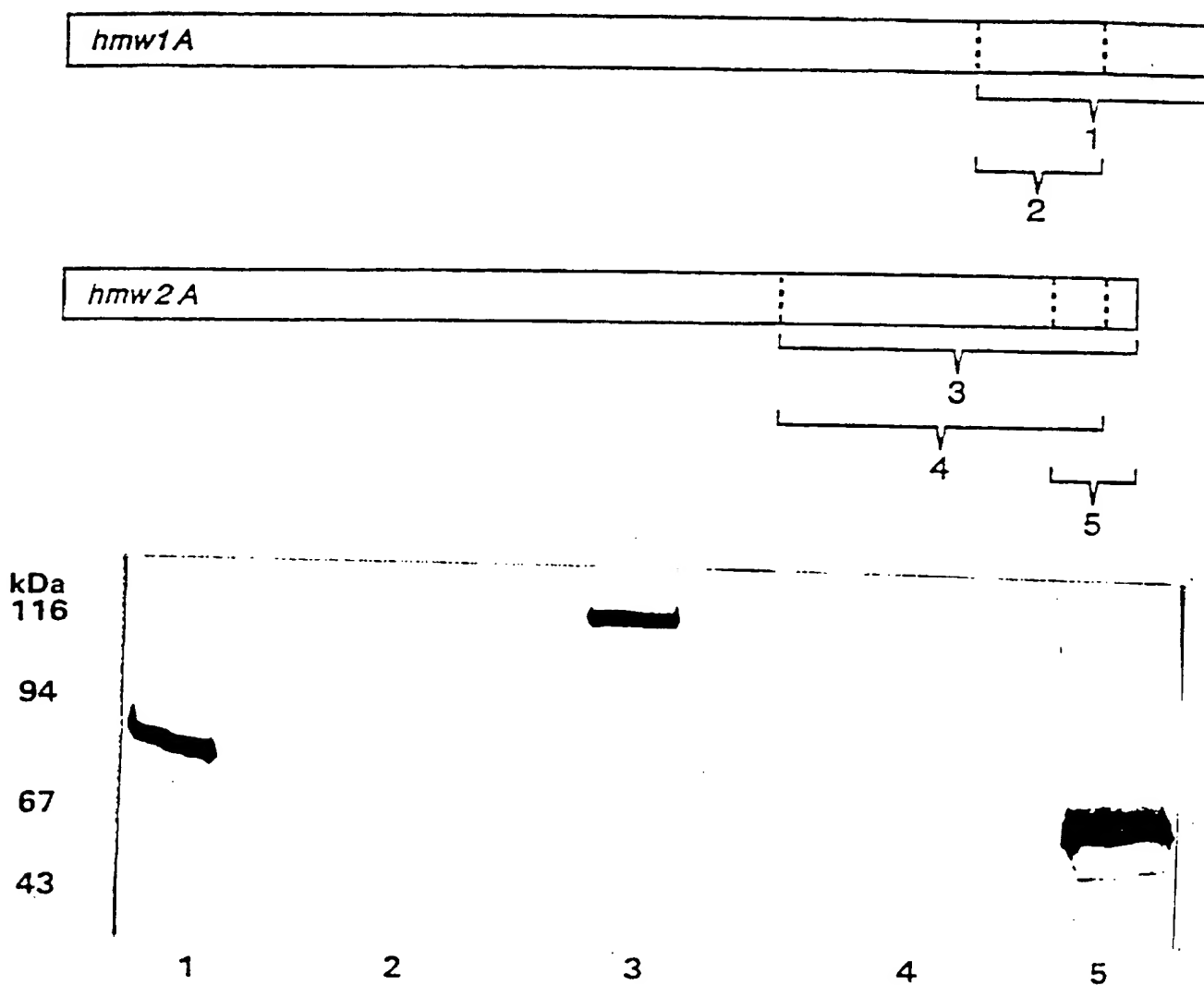


Figure 4 21

72 | 73

Western immunoblot assay with Mab 10C5 and HMW1A or HMW2A recombinant proteins

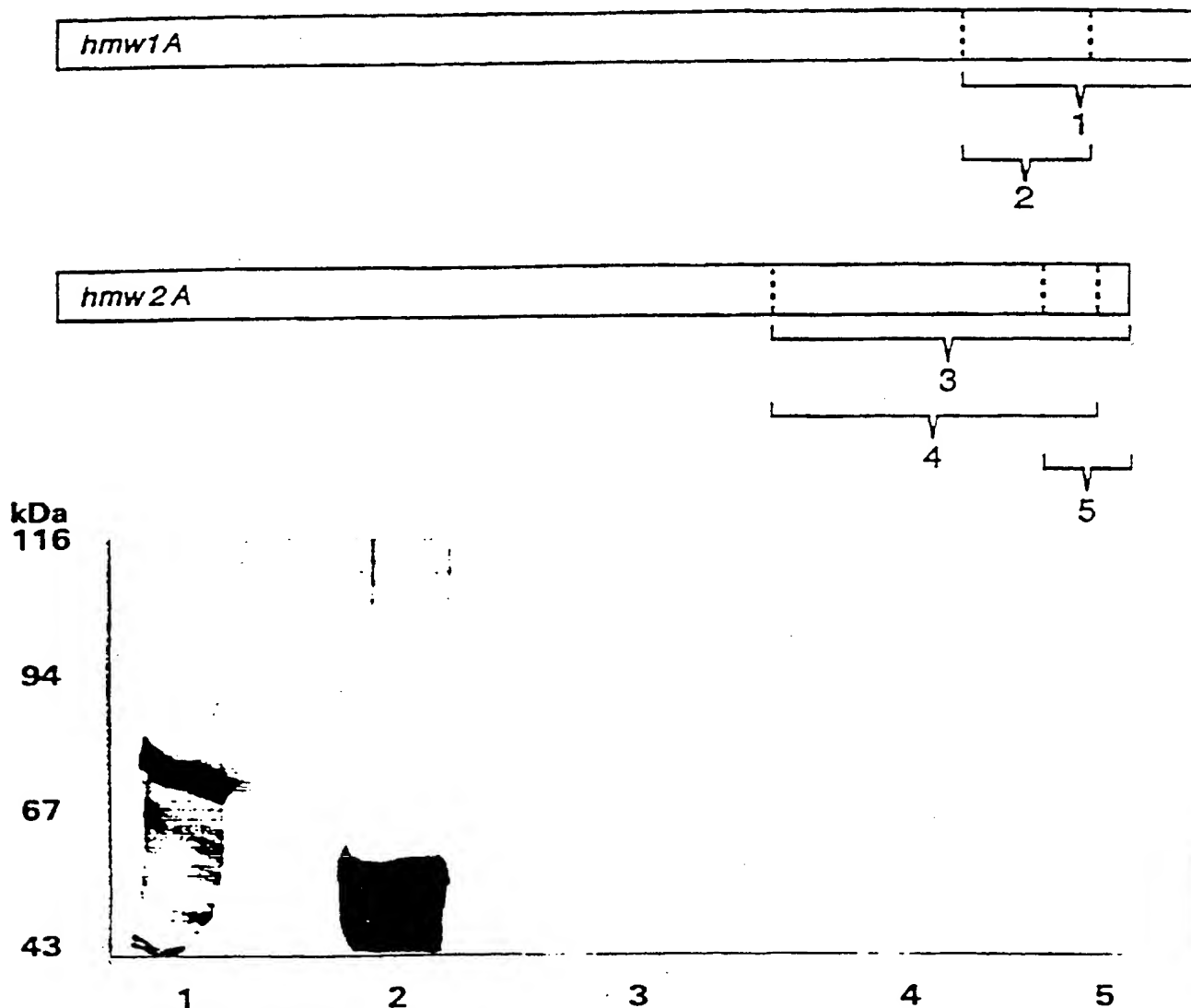


Fig 22

73 / 73

**Western immunoblot assay with Mab AD6 and
ten unrelated nontypable *Haemophilus influenzae***

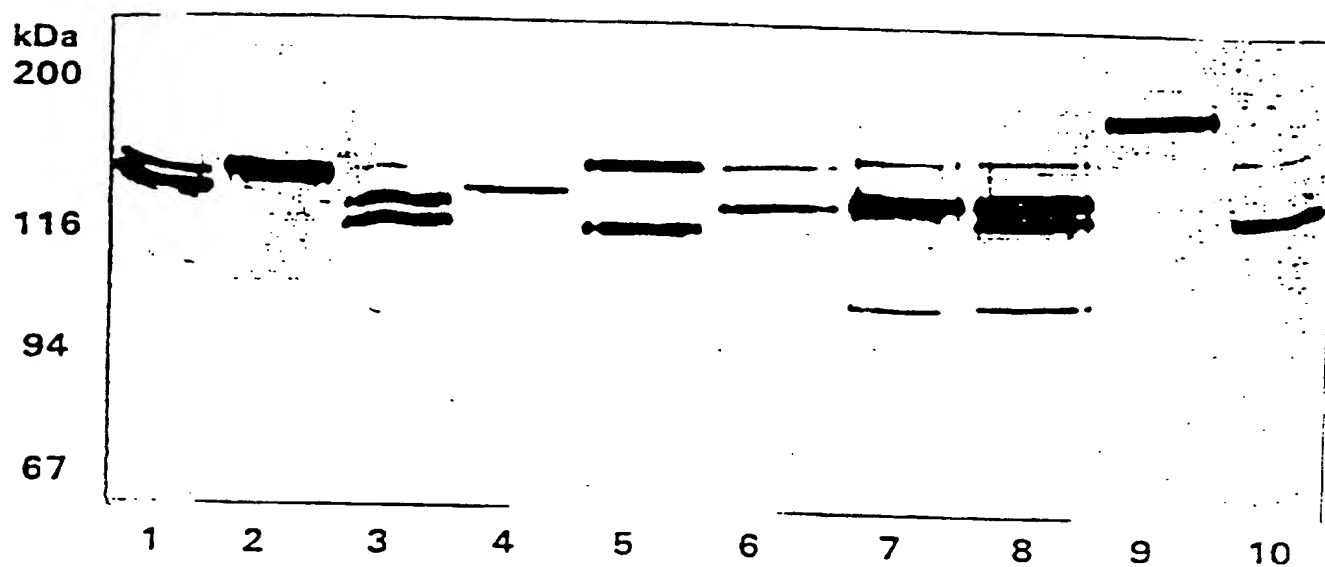
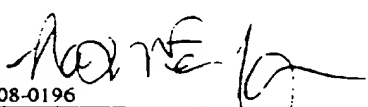


Figure 5 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04707

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : C07H 21/02, 21/04; C12P 21/06; A61K 39/102 US CL : 536/23.1, 23.4, 23.7, 24.3, 24.33; 435/69.1; 424/256.1 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 536/23.1, 23.4, 23.7, 24.3, 24.33; 435/69.1; 424/256.1 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS, DIALOG, CAS, MEDLINE, BIOSIS, MPSRCH search terms: haemophilus influenzae, h. influenzae, high molecular weight, hmw		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/19090 A1 (BARENKAMP) 30 September 1993, entire document.	1-4
X	BARENKAMP et al. Cloning, Expression, and DNA Sequence Analysis of Genes Encoding Nontypeable <i>Haemophilus influenzae</i> High-Molecular-Weight Surface-Exposed Proteins Related to Filamentous Hemagglutinin of <i>Bordetella pertussis</i> . Infection and Immunity. April 1992, Volume 60, No. 4, pages 1302-1313, entire document.	2-4 ----- 1
Y		
X	WO 94/21290 A1 (BARENKAMP) 29 September 1994, entire document.	1-4
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
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P document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 14 MAY 1997	Date of mailing of the international search report 10 JUN 1997	
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JENNIFER SHAVER  Telephone No. (703) 308-0196	

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